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STEAM AND CHEMICAL SOIL DISINFECTION WITH SPECIAL REFERENCE TO POTATO WART[1]

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INTRODUCTION

Soil disinfection studies were begun in 1919 in order to find a method by which the potato-wart disease caused by *Synchytrium endobioticum* (Schilb.) Perc. could be eradicated from infected soil. At that time little was known of the susceptibility of important potato varieties grown in the United States or of the probable rate of spread of the disease. With a total known infected area of only about 100 acres in Pennsylvania, Maryland, and West Virginia (fig. 1) largely in the mountainous coal-mining regions, treatment of the entire area would have been feasible if a cheap, effective method of treatment had been found and the disease had proved destructive to a large number of our commercial varieties. It should be borne in mind that any method of soil treatment that would kill the thick-walled wart sporangia would almost certainly be too expensive to use even on small outlying areas unless it insured actual extermination of the wart organism—nearly perfect control would not justify such heavy expenditures.

In order to determine the susceptibility of potato varieties and of related plants and to study the behavior of the disease in America, as well as to test possible methods of eradication, a field station was established at Freeland, Pa., in 1919 and work was carried on during that and several succeeding years.

Fortunately a majority of our commercial potato varieties seem to be immune or highly resistant to the potato-wart disease and the disease appears to spread rather slowly. Quarantines and the growing of immune varieties in infected areas seem likely to eradicate the disease in time (*20, 21*).[3] Although a number of soil treatments were used with apparently entire success in the eradication experiments, the development of some fundamental principles underlying the successful use of heat and, more particularly, of chemicals in soil disinfection, and the presentation of the supporting data constitute the more important parts of this report.

[1] Received for publication June 6, 1924; issued September, 1925.

[2] These studies were made as part of a general cooperative project on potato wart between the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, the Federal Horticultural Board, United States Department of Agriculture, Pennsylvania State College, and the Pennsylvania State Department of Agriculture. Authorship of Part II should be credited to Hunt and O'Donnell, of Part IV to Marshall.

[3] Reference is made by number (italic) to "Literature cited," p. 363.

PART I.—THE EFFECT OF STEAM AND CHEMICAL SOIL TREATMENTS ON THE OCCURRENCE OF POTATO WART

Synchytrium endobioticum has no mycelium, producing only sporangia and swarm spores. The latter infect potato stolons and tubers, usually only at the eyes, producing the cauliflower-like outgrowths or excrescences commonly called "warts." These warts soon decay, leaving the numerous sporangia to become incorporated in the soil and to spread by cultivation, water and air currents, tubers, tools, the feet, or other agents (*22*). According to apparently authentic reports the sporangia may remain viable in the soil for many years. Soil disinfection, if practicable and effective, would immediately remove the possible sources of infection.

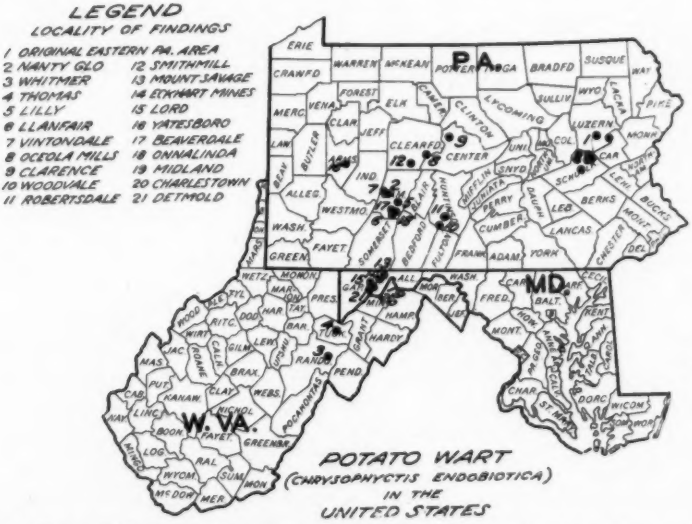


FIG. 1.—Known distribution of potato wart in the United States in 1921. Field experiments were carried out near Freeland, Pa., in the large infected area in eastern Pennsylvania

The soil disinfection experiments carried out have followed two main lines of attack: (1) The use of steam by the inverted-pan method, either alone or in conjunction with a chemical; (2) the use of chemicals.

THE STEAM-PAN TREATMENTS

Historical

Gilbert (*4*) seems to have been the first to try the inverted steam-pan method of soil disinfection for controlling a plant disease. In testing methods of disinfecting tobacco-seed beds for the control of root rot (*Thielavia basicola*), Gilbert used the inverted steam-pan on three plots, one "treated for a half hour after the soil had reached a temperature of 200° F. as indicated by a soil thermometer inserted 6 to 8 inches below the surface of the soil, 'a second' was treated one hour at a temperature of 175° 'and the third' one and one-half hours

at a temperature of 150°. The second of these plots gave the best results, being almost entirely free from disease." On the basis of his experiments Gilbert recommends that soil to be disinfected should be spaded up and thoroughly pulverized and steam applied by the inverted-pan method. "The steam should be kept at as high a pressure as possible, 80 to 100 pounds being best, and the treatment should continue for one to two hours, depending on the pressure maintained."

Numerous investigators have obtained results very similar to those of Gilbert, and the use of the steam-pan treatment of seed beds for the control of soil-borne diseases has become common practice in many places. Formaldehyde drenching has also been a common treatment for seed beds. The strength and quantity of formaldehyde solution recommended varies considerably. Gilbert recommends "1 part of commercial formalin to 150 to 200 parts of water, three-fourths to 1 gallon of this solution being used to the square foot of bed space."

Both steam and formaldehyde treatments had been used with such success in this country that Kunkel and Broadbent, who in 1919 experimented with possible methods of exterminating potato wart, undertook a retesting of these methods, notwithstanding the reported lack of success with them in England. They also tried combinations of the two treatments. In 1919, field work on this project was carried out by Broadbent [5] in heavily infected soil near Freeland, Pa. Broadbent's data show that his steam treatments varied from 25 minutes at 85 pounds gauge pressure to 175 minutes at 105 pounds pressure. No wart infection appeared in plots treated for 85 minutes at 90 pounds pressure, but occurred in a number of plots steamed 70 minutes. Plots which were sprinkled with 0.41 per cent (or stronger) formaldehyde at the rate of 0.59 pint per square foot and immediately steamed for 30 minutes at 90 pounds pressure were free from wart. The treatments with formaldehyde alone were unsuccessful.

1920 Steam-Pan Treatments

Steaming operations were begun at Freeland, Pa., on May 26, 1920. Thirty-one plots were treated up to and including June 8. The remaining seven plots were treated during the period June 25 to July 2. The garden used for the steam-pan experiments in 1920 adjoined the one used for similar work in 1919, the latter in turn adjoined the garden from which the disease was first reported. Freeland is at the southern boundary of the glacial advance and the soil contains elements of the Volusia and DeKalb types. Both are largely silt loams with a tendency to be acid. The gardens of the coal miners of that locality are largely a conglomeration of soil, ashes, coal-dust, cinders, and débris of any kind, and the steam-treated garden was no exception.

The source of steam for the experiments of both years was a large high-pressure (135 to 140 pounds) main used for pumping, hoisting, and other work about the mines. Steam was conducted nearly 200 feet from the main through a ¾-inch pipe, a 20-foot length of steam hose serving to connect this pipe with the steam-pans. The steam-pans were those used in 1919, one covering 5 by 9 feet and the other 6 by 9 feet. These are merely shallow open boxes; the sides are made of 2 by 6 inch material from the lower edge of which an iron

[5] Unpublished report of field work in soil disinfection to exterminate potato wart, 1919.

strip projects to insure a steam-tight contact with the soil. The top consists of two layers of thin tongue-and-groove boards with a layer of tar paper between. Handles are bolted to the sides. The steam inlet is centrally located in the top of the pan, and a metal plate fastened just below the opening serves to deflect the steam in all directions parallel to the soil surface. A steam-pressure gauge was attached to the steam supply pipe about 40 feet from the pan. The pressure was read with the steam to the pan shut off. The duration of steaming is expressed as the time elapsing between the opening and closing of the valve at the gauge. The pan was left in place for 30 minutes after steam was shut off, thus prolonging the heating effect. By using electric resistance bulbs the rise and fall of temperatures at different depths in the soil under the steam pan was followed. A summary of these data and of the conditions under which the treatments were carried out is presented later. It seems likely from later tests that the amount of steam supplied to the steam pan was not accurately measured by the gauge pressure. The temperatures obtained were not always uniform. This may explain the lack of complete disinfection in some of the plots which were steamed for long periods.

When the pans were moved they were set down so as to overlap by at least 6 inches the area previously steamed. Usually two pan areas which were similarly treated constituted a plot. In a few cases, one or three pan areas made up a plot. As soon as the steaming of a plot was completed, a trench a foot wide and 3 inches deep was carefully dug around it. Stakes were then driven into the soil at each corner. Boards were nailed to these to form sides which projected above the soil level and slightly below the bottom of the trench. The trench and board frames were provided to prevent contamination of the plots by surface water. A 2-foot wire-netting fence was attached above these base boards and a wire-netting top was fitted on in order to exclude birds and quadrupeds which might otherwise contaminate the plot. Great care was taken to have boots, tools, lumber, and other supplies used about the plots sterile so that no infected material reached the steamed soil during the progress of the work. The wind blew outside soil across the steam plots on one or two occasions. It was found necessary to sprinkle the surrounding area lightly with water to prevent this. These experiments in 1920 were planned to retest the steam and formaldehyde treatments which the results obtained in 1919 indicated to be of greatest promise. In view of the striking results obtained in 1919 by the application of dilute solutions of mercuric chloride, certain plots received a combination of mercury drench and steam treatment.

The steam-pan treatments in 1920, using steam alone, varied from 70 to 115 minutes, with the gauge showing 90 to 95 pounds pressure, and from 12 to 25 minutes at 140 pounds pressure. The steam-pan treatments which followed the application of formaldehyde at the rate of ½ pint per square foot ranged from 25 to 40 minutes of steaming at 90 to 95 pounds pressure. The steam-pan treatments which followed the application of mercuric chloride each lasted for 25 minutes with steam at 90 to 95 pounds gauge pressure. Mercuric chloride was applied at the rate of 80 c. c. of a 1 to 20,000 solution to 80 c. c. of a 1 to 500 solution per square foot, and in one instance at the rate of 160 c. c. of a 1 to 400 solution per square foot.

Results of Steam-Pan Treatments

RESULTS IN 1920

Through an error on the part of the seed house which furnished them, the potatoes used in planting most of the plots in 1920 were of a wart-immune variety and consequently very meager results were obtained. Nine plots were, however, planted with susceptible varieties. Of these, five became infected with wart, as did also two plots which were left untreated as controls Two plots in which wart developed had been treated with 1 per cent formaldehyde (½ pint per square foot) followed by steam at 90 to 95 pounds pressure for 25 minutes; one with 1.5 per cent formaldehyde followed by steam for 40 minutes; and one treated with steam alone for 12 minutes at 140 pounds pressure. The four plots which were free from wart at harvest had been treated as follows: One plot with 160 c. c. per square foot of a 1 to 400 solution of mercuric chloride followed by steam for 25 minutes; one with 1½ per cent formaldehyde and steamed for 25 minutes; one with steam alone at 140 pounds pressure for 25 minutes; and one with steam alone for 75 minutes at 90 to 95 pounds pressure. The results practically eliminated the possibility of using a short steaming period following treatment of the soil with formaldehyde, since four of the five plots receiving such treatments and planted to susceptible varieties were found to have produced warted potatoes.

RESULTS IN 1921

In 1921 the plots given steam-pan treatments in 1920 were planted with varieties of potatoes known to be susceptible to wart. Figure 2 presents a diagram showing the treatments given, the relationship of the plots to each other, and indicates the occurrence of wart in 1921. Table I summarizes the results obtained.

Three of the 6 plots given 85 minutes or more of steaming at 90 pounds pressure showed wart, as did a large part of the steam-formaldehyde plots and all of the steam-mercuric chloride plots. Steaming for 85 minutes should heat every wart spore in the soil to a temperature of 90° C. or more and keep it hot for some minutes. These results indicate that the potato wart organism is very resistant to heat. It may be that only the wart spores in the upper 4 or 5 inches of soil were killed by the treatments. Potatoes were planted the first year without stirring the soil to more than this depth, whereas the plots were spaded to a depth of 7 or 8 inches before planting the second season. This may explain in part at least the difference between the results Broadbent obtained from planting the same year the treatments were given, and the results cited above, which were derived principally from plantings made the year following treatment. This view is borne out by the fact that of the nine plots treated in 1920 and planted to susceptible varieties in 1920 all but the one given 75 minutes of steaming showed wart in 1921, whereas in 1920 four of these plots were wart free.

One of the plots which was steamed for 85 minutes and was free from wart at harvest lay between two plots which showed over 60 per cent of wart-infected plants. This would indicate that under favorable conditions this treatment is effective, even in badly infected soil. It will be noted that steaming at the maximum pressure

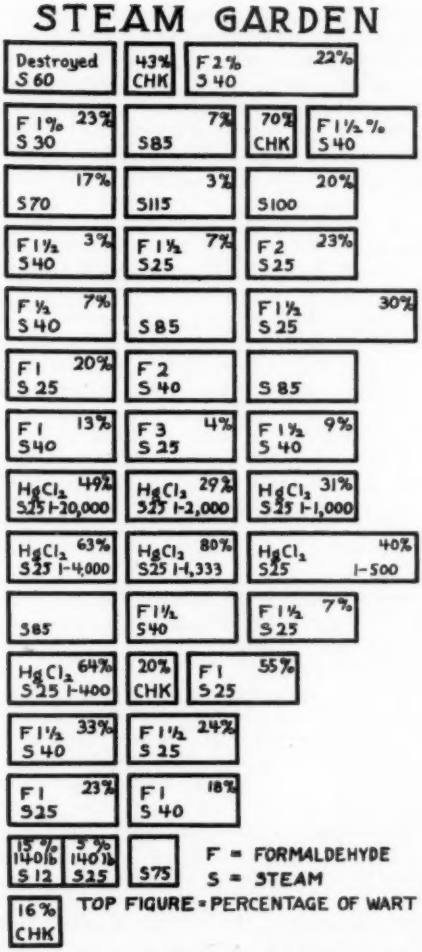


FIG. 2.—Diagram of the plots treated with the steam pan in 1920, showing relative size and arrangement of plots. The length of steaming periods is shown in minutes (e. g., S 60=steam allowed to flow into pan for 60 minutes). The steam pressure was 90 to 95 pounds, except for the two plots where 140 pounds pressure is indicated. The steam pan was removed 30 minutes after steam was shut off. Where ½ pint of formaldehyde solution per square foot was applied previous to steaming its strength is indicated (e. g., F 1½ or F 1½ %=1½ per cent of commercial formaldehyde). Mercuric chloride solutions were used at the strengths indicated. The applications were at the rate of 80 c. c. per square foot except that the 1 to 400 solution was applied at the rate of 160 c. c. per square foot. The upper numeral (when present) is the percentage of the hills of potatoes showing wart in 1921. (Building operations caused the destruction of one plot before any results were secured)

TABLE I.—*Presence of wart in 1921 following steam and combination treatments in 1920* [a]

Wart absent					Wart present						
Formaldehyde		Steam		Plots	Formaldehyde		Mercuric chloride		Steam		Plots
Strength	Amt. per sq. ft.	Pressure	Time		Strength	Amt. per sq. ft.	Strength	Amt. per sq. ft.	Pressure	Time	
Per cent	*Pints*	*Pounds*	*Minutes*	*Number*	*Per cent*	*Pints*	*Per cent*	*C. c.*	*Pounds*	*Minutes*	*Number*
		90 to 95	85	3					90 to 95	115	1
		90 to 95	75	1					90 to 95	100	1
2	½	90 to 95	40	1					90 to 95	85	1
1½	½	90 to 95	40	1					90 to 95	70	1
									140	25	1
									140	12	1
					1½	½			90 to 95	40	4
					1	½			90 to 95	40	2
					½	½			90 to 95	40	1
					2	½			90 to 95	30	1
					1	½			90 to 95	30	1
					3	½			90 to 95	25	1
					2	½			90 to 95	25	1
					1½	½			90 to 95	25	4
					1	½			90 to 95	25	3
							1 to 400	100	90 to 95	25	1
							1 to 500	80	90 to 95	25	1
							1 to 1,000	80	90 to 95	25	1
							1 to 1,333	80	90 to 95	25	1
							1 to 2,000	80	90 to 95	25	1
							1 to 4,000	80	90 to 95	25	1
							1 to 20,000	80	90 to 95	25	1

[a] Commercial formaldehyde considered as 100 per cent. One plot was destroyed during winter 1920–21. English weights and measures were used when metric apparatus was not available or whenever more convenient for any reason.

(135 to 140 pounds) carried by the mains was tried. Steam at this pressure heats the soil very rapidly but the treatment is too difficult to carry out. The 12-minute steaming period was due to the bursting of the hose. An ordinary boiler can not maintain such high pressure. The combination of formaldehyde with short periods of steaming did not prove effective. Formaldehyde applied at the rate of one-half pint per square foot does not penetrate the soil more than about an inch. Steaming for 40 minutes at 90 pounds pressure can not be depended on to sterilize deeper than 3 to 4 inches. As noted before, this treatment might result in a clean crop the first year, before the deeper-lying spores are brought to the surface by cultivation. As will be noted in Table I every plot treated with both steam and mercuric chloride was found to have warted plants. The quantity of mercuric chloride solution applied penetrated only a fraction of an inch and the steam treatment added would not be effective to a depth greater than 2 to 3 inches.

The results of the 1920 steam-pan experiments seem to eliminate the steam-formaldehyde and the steam-mercuric chloride treatments, inasmuch as they were too expensive to use unless they insured absolute eradication. The results also cast grave doubts on the dependability of steaming alone for 85 minutes at 90 pounds pressure. The steam-pan treatments as carried out were not effective. The failure of the longer treatments was probably due to the fact that the method of controlling the amount of steam supplied was not accurate, as determined later. However, much of the ground could not be readily treated by the steam-pan method.

Potato wart has been found in over 800 gardens. These vary in size from a few square feet to one-fourth of an acre. Furthermore, the gardens are usually irregularly shaped and are likely to be rendered more difficult to treat by the presence of walks, outbuildings, trees, clothesline posts, ditches, or rock outcroppings. It is practically impossible to treat all the soil next the fences and buildings. In considering the possibility of exterminating potato wart it must be borne in mind that the pathogene is undoubtedly present to a greater or less extent in soil surrounding the gardens in which its presence has actually been established. Spores are easily carried on the feet or on implements from one part of the yard to another and also to adjacent streets, alleys, and yards. The disease was often particularly severe along rear walks where potato peelings had been thrown from the kitchen door. Diseased potatoes were sometimes stored in earthen-floored cellars. Besides these practical difficulties in the use of steam-pan treatments, it is a rather slow and expensive method. Because of the difficulties involved in steam-pan treatments, it seemed desirable to restrict further studies on soil disinfection to tests of chemicals, including a study of the principles underlying their use.

DISINFECTION OF SOIL BY VARIOUS CHEMICAL TREATMENTS

Historical

The use of chemicals by prior workers for sterilizing soil has, like that of steam, been practically limited to the treatment of greenhouse benches or to seed beds. In either case control rather than eradication was the object sought. For seed beds particularly, only surface sterilization was necessary, as in many cases the control of damping off was the principal objective. Halstead (*6*) carried out an extensive series of experiments by treating soil with ashes, benzine, Bordeaux, calcium carbonate, carbon bisulphide, copper sulphate, corrosive sublimate, creolin, cupram, formalin, gas lime, kainit, kerosene, lime, manure, oxalic acid, sodium carbonate, sodium chloride, sulphate of ammonium, sulphate of potassium, sulphide of ammonium, sulphide of potassium, sulphur, and sulphuric acid. These experiments showed that sulphur was a valuable preventive of potato scab; that it was a preventive of soil rot in sweet potatoes, especially when used with kainit; and that air-slaked lime was a practical remedy for club root in turnips. The other chemicals failed to show promise as soil fungicides. As control, not extermination, of the diseases was the object of the experiments, the amount of chemical used was relatively small in all cases. The maximum applications of mercuric chloride, for example, were 1 gram per square foot dry, or 1 pint of a 1 to 1,000 solution; of sulphur $\frac{2}{5}$ ounce; of calcium carbonate 1 ounce; and of kerosene $\frac{1}{25}$ ounce per square foot. Only surface or partial sterilization was possible with the amounts used.

For the control of tobacco root rot, formaldehyde drenching was found by Gilbert (*4*) to give reasonably satisfactory results, though less effective as well as more expensive than the steam treatments tried. Hartley and others (*7, 8, 9*) have done considerable work on methods of controlling damping off among coniferous seedlings. They found that the chemicals which gave the best results depended on the character of the soil and the organism or organisms involved.

Efforts of European investigators to control or eradicate potato wart by soil-disinfection methods have failed. Among the chemicals

tried were formaldehyde, lime, bichloride of mercury, and sulphur. Malthouse (*12, 13, 14*) and others in England, and Schaffnit and Voss (*19*) in Germany tried a wide range of fungicides in seed and soil treatments without finding any method that gave consistent control. Malthouse, for example, found that copper sulphate used as a summer dressing almost eradicated the disease in 1909, but failed to check it the following year. Eriksson (*3*) reported successful control of potato wart with 1 per cent formaldehyde used at the rate of 1 liter per square meter. Experiments in England (*1*) based on the results of Eriksson, but on a larger scale, failed to show any control where 1 per cent formaldehyde was used at the rate of 2 gallons per square yard. Every hill, in both treated and untreated soil, showed wart.

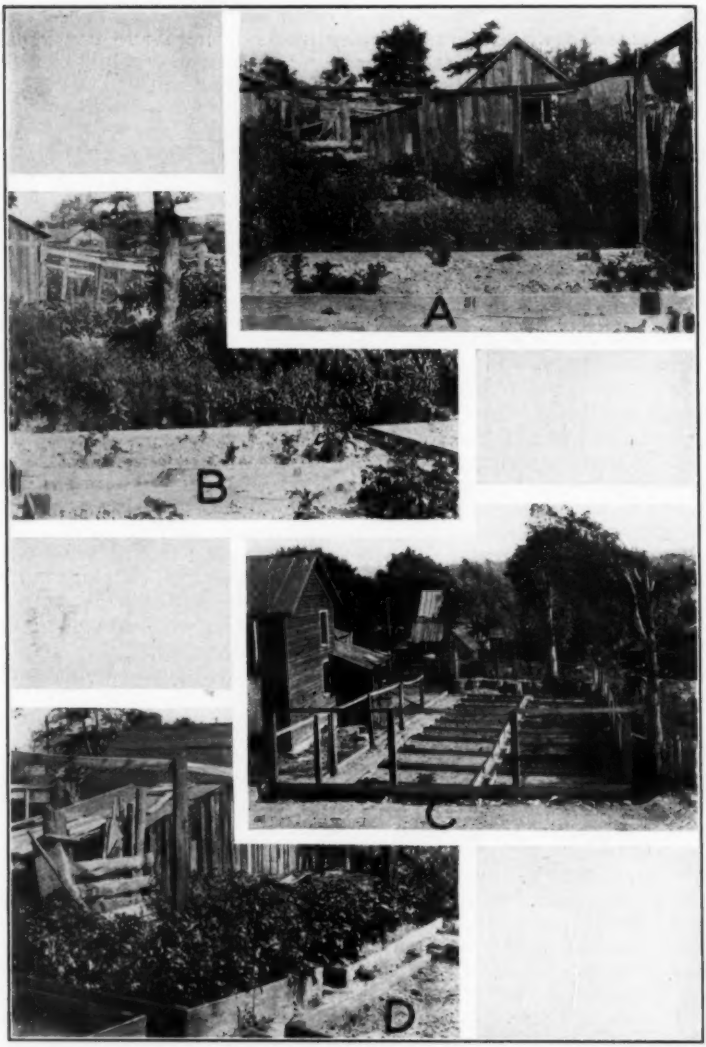
In 1919 Kunkel and Broadbent [6] tested formaldehyde on 5 by 17 feet plots, all applications being made at the rate of 1.08 pints of solution per square foot, the strength of solution varying from 0.41 to 3.28 per cent of commercial formaldehyde. One-half of the plots were treated with cold solutions to compare with duplicate plots treated with hot (97° C.) solutions. All the plots were planted to wart-susceptible potatoes. Wart was found in each of these plots at digging time. In addition, tests were made with 10-inch earthenware pots. In these experiments the following treatments were effective in eliminating wart: Autoclaving pots filled with infected soil for 15 minutes at 15 pounds pressure; bichloride of mercury, 1 to 20,000 or stronger, applied at the rate of 40 c. c. per pot (⅓ cubic foot of soil). The following treatments failed to eliminate wart: Copper sulphate, 1 to 1,000 or weaker, applied at the rate of 40 c. c. per pot; sulphuric acid (concentrated commercial) 1 to 300, at the rate of 625 c. c. per pot; copper sulphate crystals, unweighed amounts (some pots received four uncrushed crystals of copper sulphate, averaging nearly 0.5 inch in diameter and others an approximately equal amount of crushed crystals).

Chemical Treatments, Spring of 1920

In the spring of 1920, experiments were planned to test further the results of Kunkel and Broadbent as well as to try a number of new treatments. Because of the scarcity of water in much of the wart-infected area, this factor must be taken into account in chemical treatments which require an extensive use of water solutions. The liquid treatments in the spring of 1920 were therefore planned with 2 quarts per square foot as a maximum application. The plots were 6 by 12 feet in size and located in the garden in which wart was first reported and in nearby wart-infected gardens. The plots were protected from contamination by trenching and boarding up as in the steam-pan garden, but no cages were put over the individual plots. A wire-netting fence surrounded the entire group of plots in each case (pl. 1, C).

During the summer a special study of the penetration of chemicals into the soil was begun. Chemical tests of the soil from various depths in the treated areas indicated that although an application of two quarts of formaldehyde solution per square foot might in some instances penetrate the soil to a depth of 8 inches it could not be depended upon to reach a depth of more than 4 or 5 inches. The

[6] From unpublished manuscript and data.



A.—Potatoes in a plot treated with calcium chlcride, ½ pound per square foot, before planting time
B.—Potatoes in a plot treated with 1 per cent $HgCl_2$, 1 gallon per square foot, before planting time. The plot in the foreground was treated with formaldehyde
C.—General view of plots 1 to 20 before treatment in 1921, showing trenches, frames, and fence used to reduce possibility of reinfection by surface water or animals
D.—Potatoes in plots treated with Bordeaux mixture before planting time. Plot on left treated with Bordeaux 16–16–50, 1 quart per square foot, plot on right with Bordeaux 8–8–50, 2 quarts per square foot. Small parts of barren plots treated with copper sulphate and sodium fluoride respectively shown in the foreground

failure of water solutions of chemicals in such amounts was therefore not cause for surprise. Like the plots in the steam-pan treatments, most of the chemical plots were planted to an immune variety in 1920 because of a mixture of seed, so that the results of the treatments were not obtained until 1921. These spring treatments in 1920, all of which failed to eliminate wart, included: Formaldehyde, applied so as to furnish all possible combinations of 1 and 2 per cent solutions, used hot and cold, at the rate of 1 and 2 quarts per square foot; bichloride of mercury, from ½ pint per square foot of 1 to 32,000 to 2 quarts of 1 to 250; chloride of lime, ½ pint per square foot of solutions made by dissolving 12 ounces or 24 ounces in 4½ gallons of water; a commercial "potato powder" (compounded from white arsenic, lime, and copper sulphate), 1 pound to 3 gallons of water and 2 pounds to 3 gallons, ⅔ pint per square foot; and Bordeaux mixture, 8–8–50 and 16–16–50, ⅔ pint per square foot.

Chemical Treatments, Fall of 1920

Notwithstanding the fact that the penetration tests indicated that applications of 2 quarts per square foot would immediately penetrate only 4 to 5 inches, treatments made in the fall of 1920 were confined to applications of 1 pint or less per square foot or to dry chemicals. This was done because of the difficulty that would undoubtedly be experienced in obtaining sufficient water if heavy applications were attempted on a large scale. Part of these treatments were made during rain and snow storms with the hope that the rains would carry the chemicals into the soil to a sufficient depth to insure complete sterilization. While rainfall can undoubtedly take the place of some of the water that would otherwise be required, it has not been determined to what extent this is possible. Where rainfall is reasonably heavy it is a potential source of most of the water required, as 1 inch of rain is approximately equal to 5 pints per square foot.

The plots treated with chemicals in the fall of 1920 were spaded up and planted to wart-susceptible potatoes in the spring of 1921. The results are tabulated in Table II. As shown in this table, two plots were treated with a lime-sulphur solution at the rate of 1 pint per square foot, consisting of one part of commercial solution, testing 33° Baumé, to two parts of water, and permitted very little growth. The few stunted plants that appeared bore no tubers and showed no sign of wart. No wart occurred in plots treated with crude carbolic acid, or with denatured alcohol at the rate of $\frac{1}{10}$ gallon per square foot. A plot treated with kerosene at the rate of 1 pint per square foot gave striking results. The odor of kerosene was quite evident when this plot was dug nearly a year after treatment, and the tubers were absolutely free from wart, scab, and black scurf. The plot adjoining it on the upper side had 47 per cent of wart and the tubers in nearly all plots were more or less severely infected with scab and scurf. While the plot was evidently at the edge of the infected area and may have contained relatively few wart spores, the results were such as to warrant further tests of kerosene. The plants in plots treated with creosote, potassium permanganate, sugar, and sulphur were free from wart at harvest, but the soil in them was probably only lightly, if at all, infected. Plots treated with bichloride of mercury and salt (both dry and in solution), with Bordeaux, and with chloride of lime, showed a large percentage of wart except for one Bordeaux plot in lightly infected soil. This garden was said to have

been unused for 20 years prior to 1918. Potatoes planted in it that year developed wart, although the seed is said to have been clean. The source and distribution of the disease in this garden were therefore unknown, but evidently infection was not general. No further test plantings were made.

TABLE II.—*Results of chemical treatments made in the fall of 1920 on plots planted to wart-susceptible potatoes in 1921*

Wart present at harvest (per cent)	Chemical used	Application		Plot [a] No.
		Formula	Rate per square foot	
0	Alcohol	70 per cent	1 pint	14
27	Mercuric chloride+sodium chloride	1 to 4½	9½ grams	10
93 to 100	Mercuric chloride+sodium chloride+water.	400 grams, 4 pounds to 25 gallons.	1 pint	7, 8
0 to 100	Bordeaux	16–16–50 or 8–8–50	do	5, 6, 16
0	Carbolic acid (crude)		⅛ gallon	3
47 to 48	Chloride of lime	50–50	1 pint	11, 12
[b] 0	Creosote		½ gallon	22
0	Kerosene		1 pint	13, 15
0	Lime-sulphur	1 to 3, 1 to 6, 1 to 12	do	1, 2, 19, 21
[b] 0	Potassium permanganate	1 to 25	do	20
[b] 0	Sugar		2 ounces	24
[b] 0	Sulphur		¼ or ½ pound	17, 18, 23
37 to 90	Checks			4, 9

[a] Plots were 5 by 10 feet. [b] Soil probably very lightly infected, if at all, in these plots.

East

North	2	4	6	8	10	12	14	16	18	20	22	24	South
	1	3	5	7	9	11	13	15	17	19	21	23	

West

CHEMICAL TREATMENTS IN 1921

PLOT EXPERIMENTS

In the spring of 1921 four gardens with space for 100 plots, each 5 by 10 feet, were secured at Sandy Run, near Freeland, Pa., for a more comprehensive test of chemical soil sterilization. Since the preliminary tests of chemical penetration indicated that heavy applications were necessary to secure penetration, and since no dependence could be placed on rainfall in the spring, these treatments were planned for heavy applications of solution or for dry chemicals. Many of the treatments planned were so drastic as to make it almost certain that they would prevent the growth of crops for a time. It was hoped that an effective treatment could be found that would not prove injurious to crops, but wart extermination was necessarily the primary consideration. Since no effective chemical soil-sterilization treatment for this disease was known, any successful treatment, however drastic or expensive, would be a considerable advance and offer a basis for further study.

RESULTS IN 1921, 1922, AND 1923.—The details of the chemical soil treatments tested in 1921, including the strength, amount, form, and method of application, together with the results secured in wart control, are given in Table III. The diagram on Figure 3 shows the relationship of the plots to each other and shows the occurrence of wart in plots and checks in 1921 and 1922.

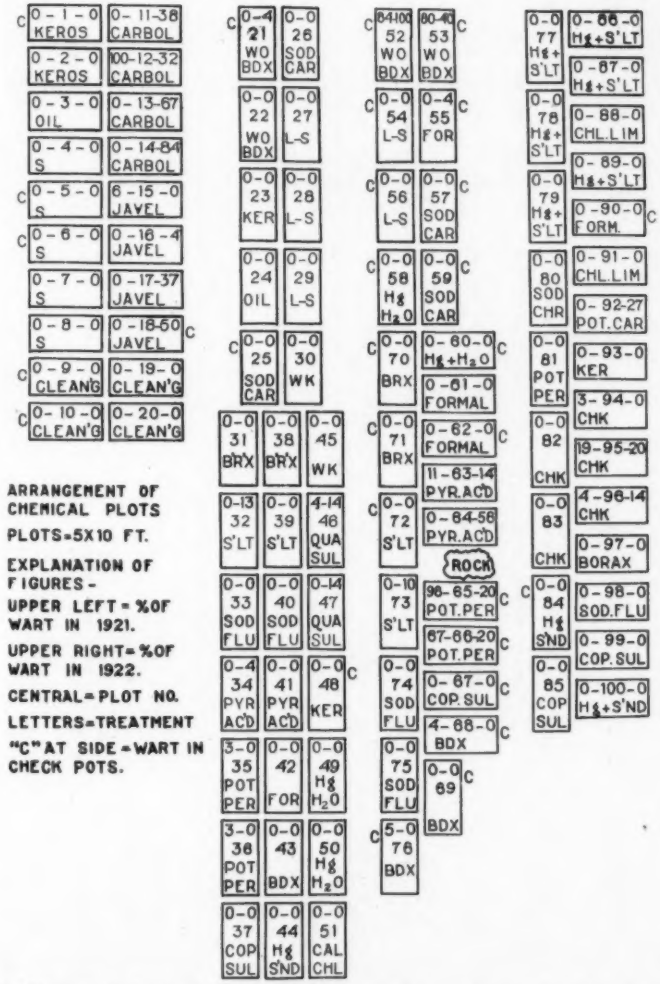


FIG. 3.—Diagrammatic drawing showing arrangement of plots in each of the four gardens used, together with the chemicals used and the results for two years. The various chemicals are indicated as follows:

Bichloride of mercury, Hg; Borax, Brx; Bordeaux mixture, Bdx; Woburn Bordeaux, Wo Bdx; Carbolic acid, Carbol; Chloride of lime, Cal Chl or Chl lim; Cleaning solution, Clean'g; Copper sulphate, Cop Sul; Formaldehyde, For, Form, or Formal; Javelle water, Javel; Kerosene, Ker or Keros; Lime-sulphur, L-S; Crude oil, oil; Potassium carbonate, Pot Carb; Potassium permanganate, Pot Per; Pyroligneous acid, Pyr acd; Qua-Sul, Qua Sul; Sodium carbonate, Sod car; Sodium chloride, Slt; Sodium chromate, Sod Chr; Sodium fluoride, Sod Flu; Sulphur, S; commercial weed killer, W K

Sand, sand and water, H_2O, were used with bichloride of mercury in some plots. Chk=Check plot. For formulae and applications see Table III, p. 314. Before the plots were treated, two pots were filled with composite soil from each. *C* at the side of a plot indicates that wart was produced on the potato plant grown in one or both check pots for that plot. For a discussion of the value of these check pots see p. 316 and Table IV

TABLE III.—*Control of potato wart during three successive years, following chemical treatments applied in 1921*

Per cent of hills showing wart			Exposure of plot to wart	Cost per acre [a]	Chemicals used	Formula	Amount used per square foot	Plot No.
1921	1922	1923						
0	0	0	[c] 1 foot, C, 1 foot	$1,900	Mercuric chloride+water	2 pounds to 25 gallons	½ gallon	[d] 50, 58, 81
[b] 0	[b] 0	0	1 foot, C	....do	....do	....do	1 gallon	49, 60
0	0	0	1 foot	....	Mercuric chloride+sand	1,000 grams to 12½ pounds	20 grams mercuric chloride.	44
0	[b] 0	0	C	....do	....do	8 pounds to 50 pounds	....do	84
0	0	0	6 feet	....do	....do	2 pounds to 50 pounds	....do	100
[b] 0	0	0	6 feet, 1 foot	....	Mercuric chloride+sodium chloride +water.	2 to 50 to 50	1 gallon	78, 89
[b] 0	0	0	1 foot, 12 feet	....do	....do	4 to 25 to 50	½ gallon	79, 87
[b] 0	0	0	15 feet, 18 feet	....do	....do	....do	1 gallon	77, 86
[b] 0	0, [b] 0, [b] 0	0, 83, 0	1 foot, C, 1 foot	....	Borax	As it came	½ pound	38, 71, 97
[b] 0	0, [b] 0	0, 9½	1 foot, C	....do	....do	....do	1 pound	31, 70
5	0	0	C	....	Bordeaux (regular)	8 to 8 to 50	¼ gallon	76
4	0	0	....do	....do	....do	16 to 16 to 50	....do	68
0	0	0	1 foot, C	315	....do	8 to 8 to 50	½ gallon	43, 69
0	4, 0	4, 0	C, 1 foot	....	Bordeaux (Woburn)	6 to 6 to 100	1 gallon	21, 22
84, 80	100, 40	100, 78	C, C	....do	....do	....do	2 gallons	52, 53
100, 0	32, 84	13, 14	1 foot, 1 foot	....do	Carbolic acid crystals	5 per cent	½ gallon	12, 14
0	38, 67	12½, 4	....do	....do	....do	....do	1 gallon	11, 13
0	0	32, 0	6 feet, 6 feet	....do	Chloride of lime	As it came	½ pound	51, 88
[b] 0	[b] 0	0	1 foot	2,100	....do	....do	1.6 pounds	91
[b] 0	0, 0, [b] 0	[b] 0, 0, 0	C, C, 1 foot	7,550	Cleaning solution	....	1 gallon	9, 10, 19
[b] 0	[b] 0	[b] 0	1 foot	....do	....do	....	2 gallons	20
[b] 0	[b] 0	0, [b] 0	1 foot, 1 foot	1,750	Copper sulphate	Crushed crystals	½ pound	37, 85
[b] 0	[b] 0	[b] 0	C, 1 foot	....do	....do	....do	1 pound	67, 99
0	0	0	1 foot, 1 foot	....	Formaldehyde, commercial	5 per cent	2 gallons	42, 61
0	4, 0	14, 4	C, C	....do	....do	10 per cent	....do	55, 62
0	0	0	C	24,400	....do	15 per cent	....do	90
6, [b] 0	0, 4	0	1 foot, 1 foot	....	Javelle water	[c] 40 to 10 to 25	1 gallon	15, 16
[b] 0	37, 50	12, 14	1 foot, C	....do	....do	¾ to ¼ to 25	....do	17, 18
[b] 0	[b] 0	0	1 foot	4,900	Kerosene	As it came	½ gallon	93
[b] 0	0	0	C, 1 foot	....do	....do	....do	1 gallon	1, 2
[b] 0	0	0	C	....do	....do	....do	2 gallons	48
[b] 0	0	0	6 feet	....	Kerosene and crude oil	1 gallon of each	....do	23
[b] 0	[b] 0	0	1 foot	726	Lime-sulphur	1 in 12	1 gallon	29
[b] 0	[b] 0	0	6 feet, C	....do	....do	1 in 6	½ gallon	28, 54
[b] 0	[b] 0	[b] 0	1 foot, C	....do	....do	1 in 3	....do	27, 56
[b] 0	0	0	1 foot, 1 foot	10,900	Oil, crude	As it came	1 gallon	3, 24
0	27	58	6 feet	....	Potassium carbonate	....do	⅔ pound	92
3, 90	0, 20	4, 59	1 foot, C	....	Potassium permanganate	1 to 1,000	1 gallon	36, 65
3, 67	0, 20	0, 9	....do	....do	....do	....do	2 gallons	35, 66

0, 11	0, 14	12½, 9	1 foot, 1 foot		Pyroligneous acid	As it came	½ gallon	41, 63
[b] 0	4, 56	4½, 74	do		do	do	⅔ gallon	34, 64
4	14	4	1 foot		Qua-Sul	1 to 50	½ gallon	46
0	14	4	do		do	1 to 100	1 gallon	47
[b] 0	[b] 0	0	C, C	1, 425	Sodium carbonate	As it came	2 pounds	25, 57
[b] 0	[b] 0	0	1 foot, C		do	do	3 pounds	26, 59
[b] 0	0, 10	14, 32	1 foot, 1 foot		Sodium chloride	do	½ pound	39, 73
[b] 0	13, [b] 0	12½, 100	1 foot, C		do	do	1 pound	32, 72
[b] 0	[b] 0	0	do	6, 550	Sodium chromate	1 pound to 2 gallons	2 gallons	80
[b] 0	[b] 0	[b] 0	do	3, 150	Sodium fluoride	As it came	⅔ pound	98
[b] 0	[b] 0	[b] 0	1 foot, 1 foot		do	do	½ pound	40, 75
[b] 0	[b] 0	[b] 0	do		do	do	1 pound	33, 74
0	[b] 0	0	C, 1 foot	220	Sulphur	do	⅛ pound	6, 8
0	[b] 0	[b] 0	do		do	do	¼ pound	5, 7
0	[b] 0	0	1 foot		Sulphur [e] inoculated	do	⅛ pound	4
[b] 0	[b] 0	0	do	6, 550	Weed killer [f]	10 per cent	1 gallon	30
[b] 0	[b] 0	[b] 0	do		do	20 per cent	do	45
0	0	4, 14	1 foot, 1 foot		None			82, 83
3, 19, 4	0, 20, 14	31, 65, 14	1 foot, 1 foot, 1 foot		do			94, 95, 96

Plots were 5 by 10 feet. Dry chemicals were raked or spaded in.

[a] Cost per acre as given is cost of materials only for the minimum successful application.

[b] Growth poor or none. Growth in plots receiving the same treatment often varied. Where this is indicated the notations are for plots in the same order as plot numbers in the right hand column.

[c] C.—Wart occurred in one or both of the pots filled with soil from this plot. 1 foot.—Wart occurred in a plot 1 foot away or in one or both of its check pots. Exposures are in the same order as the plot numbers in the last column.

[d] Plot 81 was treated with potassium permanganate, 2 ounces to 10 gallons, ½ gallon per square foot, previous to its treatment with mercuric chloride.

[e] The inoculated sulphur used was furnished free of charge by W. H. Martin of the New Jersey Agricultural Experiment Station.

[f] A solution of arsenite of soda, sodium chloride, and sodium carbonate.

As shown in Table III, plots receiving treatments by 14 chemicals remained wart-free through the three growing seasons 1921, 1922, and 1923. These chemicals were bichloride of mercury, Bordeaux mixture, chloride of lime, cleaning solution, copper sulphate, formaldehyde, kerosene, lime-sulphur, crude oil, sodium carbonate, sodium chromate, sodium fluoride, sulphur, and a commercial weed killer.

Many of the plots treated with these chemicals showed very little growth [7] of potatoes during the first season, 1921, some showed poor growth in 1922, and a few showed poor development in 1923. In the plots treated with sodium fluoride growth was entirely prevented during all three seasons.

The gardens used for these experiments were reported to be heavily infected with *Synchytrium endobioticum*. Soil samples from different parts of each garden were prepared in 50 per cent sugar solutions and centrifuged in order to secure visual evidence of the presence of wart sporangia. As a further check, designed to show whether or not the sporangia present were viable, two 10-inch pots were filled with composite soil from each plot before the plot was treated, and potatoes were planted in these pots the first year (1921). A "C" is shown in Figure 3, beside a number of plots indicating that wart developed in one or both of the pots filled with soil from that plot. Previous experience with pot checks had indicated definitely that they could not be depended on to give an accurate indication of the presence of wart in the plot soil. The following tabulation (Table IV) of the occurrence of wart in the check pots in 1921 as compared to the occurrence of wart in the corresponding plots brings out this point.

TABLE IV.—*Check pots as indicators of the presence of wart in plots*

Gardens containing plots	Wart present in check pots		Wart not present in check pots	
	Wart in plots	No wart in plots	Wart in plots	No wart in plots
1 to 20	1	5	7	7
21 to 51	1	2	9	19
52 to 76	11	8	3	3
77 to 100	0	2	*6	16
	13	17	*25	45

* Five of these plots were untreated

The pots were planted in 1921, only, but the plots were planted in 1921, 1922, and 1923; and no distinction is made between plots producing wart one year, two years, and three years.

It will be noted that the check pots demonstrated the presence of viable wart spores in 30 plots and only 13 of these plots produced wart. However, 16 of the remaining 17 plots received treatments which prevented wart throughout the experiments. No wart developed in either of the check pots for 70 plots, yet wart developed during one or more years in 25 of these plots. The pots demonstrated the presence of wart spores in only 30 plots, whereas they were present in at least 55 plots.

[7] The growth obtained is discussed in the section on Potato Growth Followin Certain Chemical Soil Treatments (Part IV).

It is interesting to note that no wart developed in the check pots for the 5 untreated plots. Only 2 of these 5 plots produced wart all three years. Another produced wart the first and third years. Two produced wart the third year only. While these untreated plots were in the garden showing the least wart, the results obtained serve to emphasize the necessity of continued planting of treated plots in order to make sure that the results obtained are dependable.

Treatments that are sufficiently drastic to prevent or greatly retard the growth of potato plants might be expected to kill any swarm spores produced by the wart organism as long as the inhibiting factor remains. Unless the highly resistant resting sporangia are killed or die in the meantime, it is conceivable that they might cause wart after the factor which inhibits plant growth is eliminated from the soil. Plots which showed no wart during the three growing seasons above mentioned may be grouped according to the adequacy of growth of potatoes in them to give a reliable test of the presence of viable wart spores, as follows: Plot showing no growth, that treated with sodium fluoride; plots showing inadequate growth, those treated with sulphur; plots showing doubtfully adequate growth, those treated with copper sulphate crystals, lime-sulphur solution, sodium carbonate, sodium chromate, a commercial weed killer; plots showing probably adequate growth, those treated with bichloride of mercury (in part), chloride of lime, cleaning solution, formaldehyde, kerosene; plots showing good growth, apparently adequate for a good test, those treated with Bordeaux mixture, bichloride of mercury (in part), crude oil.

RESULTS OF POT EXPERIMENTS WITH BICHLORIDE OF MERCURY AND SODIUM CHLORIDE

The efficiency of bichloride of mercury and salt solutions for killing the wart organism in the soil was tested by using treated wart-infected soil to inoculate potatoes. Two-inch glass soil tubes [8] were filled with thoroughly infected soil from one of the gardens in Freeland, Pa., the soil was compacted, a 1 per cent solution of mercuric chloride plus 5 per cent sodium chloride was made and applications added at the rate of 2 quarts per square foot. This treatment was followed almost immediately by water at the rate of 2 quarts per square foot. Chemical tests on part of the tubes showed the presence of mercury to a depth of 8 to 9 inches in the soil. At the end of three weeks, pots were partly filled with wart-free soil and potato seed pieces placed on top. Then soil was removed from the treated tubes, and from checks, an inch at a time, and sprinkled over the seed pieces and soil in the pots. The pots were then filled with wart-free soil. The potatoes made normal growth and at the end of three months were examined for the presence of wart. The 90 pots inoculated with soil from the surface to the 9-inch depth of treated tubes showed no wart. In 6 of the 29 pots inoculated with soil from below the 9-inch depth of treated tubes wart developed. In 10 of the 35 pots inoculated with untreated wart-infected soil from check tubes wart developed. A duplicate set of tubes gave almost exactly the same amounts of wart. So little of the treated soil was used in each pot

[8] These tubes were used in the study of the penetration of soil fungicides and are described on p. 331. The methods used are discussed on pp. 330-334.

that if the wart spores were not killed, any inhibitory action of the chemicals should have been overcome and the disease produced in at least some of the pots.

SUN AND AIR TREATMENTS

In planning soil treatments with a view to the extermination of the potato wart it is necessary to know something of the rate and methods of spread of the pathogen in order to know how far beyond the known limits of the infected area soil treatment will be necessary. It seems certain that wart spores are present in wind-borne soil, but the slowness with which the wart disease seems to spread in infected gardens indicates that comparatively few infections result from wind-borne soil. It was thought that relatively short exposure of spores to air and sun might be sufficient to kill them. An experiment to test this was carried out in the greenhouse during the winter of 1920–21. Wart-infected soil was spread in thin layers, ⅛ and ¼ inch deep, on wart-free soil in pots in the greenhouse and exposed to air and sunlight for from 2 to 12 days. The wart-infected soil was used as it came from the garden except that where necessary lumps were broken and stones and débris removed in order that the total thickness of the layers of soil should not exceed the specified ⅛ or ¼ inch. At the end of the exposure periods the pots were planted to potatoes by pressing a single tuber partly into the soil in each pot and covering it with wart-free soil. Of the 48 tubers planted in pots containing ⅛ inch of this wart-infected soil, 44 produced wart-infected plants. Of the 48 tubers planted in pots containing ¼ inch of wart-infected soil, 36 produced wart-infected plants. Of the 20 tubers planted in pots containing ⅛ or ¼ inch of the same lot of wart-infected soil and not exposed to air and sun, 8 produced wart-infected plants. These results seem to indicate that wart spores near the surface of freshly cultivated soil retain their vitality for some time, long enough for the soil to dry out and be blown about.

In another experiment 60 tubers were wet, rolled in wart-infected soil from the same source and exposed to air and sunlight for from 2 to 25 days. Only 3 of these tubers, exposed 2, 9, and 12 days, developed wart when planted. The fact that nearly all of the soil had fallen from these tubers before they were planted may account at least in part for the low percentage of infection obtained.

COST OF TREATMENTS

The cost of many of these treatments is much too high to warrant their use except under special circumstances.

Cost of Steam-Pan Treatments

With a minimum of overlapping a 6 by 12 foot steam pan would treat 65 square feet of soil at a time, making 670 pan areas per acre. Steaming 1 pan area every 1½ hours would accomplish the treatment of 5 pan areas each 8-hour day. The cost would approximate $15 per day divided as follows: Engineer $5, 2 laborers at $4, one-third ton of coal at $6 per ton; or $2,010 per acre. In addition there would be the cost of water, which would have to be hauled or pumped; drayage on coal; transportation of men; rental or depreciation of outfit; probability of higher labor costs, especially for engineers;

cost of a trained supervisor. The foregoing costs assume that the laborers could do all razing and replacing of fences and poles and all treatments of soil that could not be treated by the steam pan. In this work a 5 by 9 foot and a 6 by 9 foot steam pan were used, which weighed about 400 pounds each; but with several strong men or special devices, larger ones could be used. Treatment would obviously be so slow that several outfits could probably be used to advantage, greatly reducing the cost of transportation of labor and coal and possibly enabling a small crew to handle all labor for several outfits. It seems doubtful if the cost could be reduced much, if any, below $1,500 per acre.

Cost of Chemical Treatments

The applications of chemicals made in 1921 were extremely heavy, and it seems probable that positive results have been obtained with such of these chemicals as are capable of effective sterilization. The quantity of chemical used for some of the treatments could doubtless be reduced considerably without loss of efficiency. It has not been possible to carry out experiments to determine the most effective and economic quantity of each chemical used. None of the treatments have been tried on a sufficiently large scale to make possible the giving of estimates of the cost per acre of application of the materials listed. The steam used in the steam-pan experiments was furnished free of charge by a local coal company. Treatments requiring water would be relatively expensive to apply since there would be large quantities of solution to handle, and water solutions soak into the ground more or less slowly. In much of the region where potato wart occurs, the water supply is inadequate during most of the growing season and special reservoirs would have to be constructed to retain the spring run-off if water solutions were to be used on a large scale. Experiments have shown that Bordeaux mixture will penetrate the soil hardly at all and must be worked in. Kerosene and crude oil penetrate so quickly that their application would only require an accurate sprinkler or distribution system. The application of dry chemicals should be as cheap and easy as the application of fertilizers.

The prices of some of the chemicals used are extremely variable, sometimes as much as 50 per cent variation from one year to the next. Unless otherwise specified, the prices given below are those quoted in the General Schedule of Supplies of the United States Treasury Department, 1924.

Cost of materials per acre for chemical treatments preventing wart

Bichloride of mercury	$1,896.06
$5.75 for 2,500 grams. One-half gallon of 1 per cent solution per square foot. With 5 per cent of salt added to give better and more even distribution, the cost would be increased about $83.60 per acre, salt being 92 cents per hundredweight.	
Bordeaux mixture	313.55
Copper sulphate 8 cents per pound, lime (estimated) 1 cent per pound 8–8–50, ½ gallon per square foot.	
Chloride of lime	2,090.88
3 cents per pound, 1.6 pounds per square foot.	
Cleaning solution	7,550.40
Sodium chromate, 15 cents per pound. Sulphuric acid (sp. g. 1.84), 36.8 cents per gallon (5.28 cents per kilo) 10 pounds sodium chromate, 3 gallons sulphuric acid, 12 gallons water, 1 gallon per square foot.	

Copper sulphate 8 cents per pound, ½ pound per square foot.	$1,742.40
Formaldehyde $29.60 for 60 kilos. Two gallons of 15 per cent solution per square foot.	24,441.44
Kerosene 14 cents per gallon (price paid in 1921), ⅕ gallon per square foot. Results with 1 pint per square foot were very promising, so that application could probably be greatly reduced; cost at 1 pint per square foot would be $762.30.	4,878.72
Kerosene and crude oil Kerosene 14 cents, crude oil 25 cents per gallon (prices paid in 1921). One gallon of each per square foot. The "crude oil" looked like a good grade of machine oil. Crude oil can sometimes be obtained at oil wells at 5 cents per gallon.	16,988.40
Lime-sulphur Concentrated lime-sulphur solution 20 cents per gallon (estimated) 1 part in 6 parts solution, ½ gallon per square foot.	726.00
Oil, crude 25 cents per gallon (price paid in 1921) (see above under kerosene and crude oil). 1 gallon per square foot.	10,890.00
Sodium carbonate 1.62 cents per pound, 2 pounds per square foot.	1,411.34
Sodium fluoride 18 cents per pound, ⅗ pound per square foot.	3,136.32
Sulphur 4 cents per pound (estimated), 2 ounces per square foot.	217.80
Weed killer Weed killer, $1.50 per gallon (price paid in 1921) 1 gallon of 10 per cent solution per square foot.	6,534.00

The cost of materials in the foregoing list is based on the amounts actually used. Only a few of these treatments, bichloride of mercury, Bordeaux mixture, and copper sulphate, were tried in smaller quantities, but with resulting failure. It seems probable that at least some of the quantities used could be markedly reduced without prejudice to the results. Sodium fluoride at the rate used, 20 pounds per plot of 50 square feet, absolutely prevented growth during all three years. It would seem likely that a considerable reduction could be made in the rate of application of this chemical. The amount of chemical used per square foot could very likely be reduced for cleaning solution, crude oil, sodium carbonate, and weed killer. Cleaning solution is known to be a strong, rapid killing agent and in the amount used made a thin mud of the soil to the depth spaded up. Crude oil of the type used penetrates soil very readily, and sufficient depth of penetration could probably be obtained in most soils with smaller applications. Applications of sodium carbonate were very heavy, 2 pounds per square foot, and prevented growth during the first two years. Weed killer at the strengths used prevented the growth of potatoes during the first two years. It is designed to prevent the growth of green plants, and its action on wart sporangia might of course be only a temporary inhibition. Kerosene used as a fall treatment at 1 pint per square foot gave good results, but needs further testing.

SUMMARY

The only way to eradicate potato wart in infected soil quickly and effectively is by soil sterilization.

Soil may be sterilized by heat or by chemicals.

The inverted steam-pan method is the most approved method of heat sterilization of soil.

Although effective, steam-pan sterilization is slow, cumbersome, and expensive.

The application of small amounts of formaldehyde or of bichloride of mercury solutions just previous to steaming the soil can not be depended on to increase the efficiency of the treatment.

Of the 22 chemicals tried in the dilutions and amounts given, 14 produced wart-free plots during the season treated and during the two following seasons.

Six of the successful chemicals (bichloride of mercury, chloride of lime, copper sulphate, sodium carbonate, sodium fluoride, and sulphur) were used dry. Kerosene and crude oil were used undiluted. Water was used with seven of the chemicals (bichloride of mercury, Bordeaux mixture, cleaning solution, formaldehyde, lime-sulphur, sodium chromate, and a commercial weed killer).

Water is difficult to obtain in the principal region infected by potato wart, at Freeland, Pa., and this adds to the cost of applying chemicals in solution. The use of chemicals dry or applied as strong solutions during the late fall would be desirable if the amount of water required could thereby be reduced. Such data as were obtained on this point indicate that such a reduction might be possible.

Some test plots gave such poor growth of potatoes that conditions were not favorable to wart infection, and final conclusions as to the effectiveness of the chemicals used on them were therefore not possible. Sodium fluoride prevented growth all three seasons. Sulphur nearly prevented growth all three seasons. The growth in plots treated with copper sulphate crystals, lime-sulphur, sodium carbonate, sodium chromate and a commercial weed killer was such that the test of their efficacy is not considered entirely satisfactory. The growth in at least part of the plots treated with bichloride of mercury, cleaning solution, kerosene, Bordeaux mixture, and crude oil was such as to make it seem almost certain that wart would have developed if the treatment had not been effective.

The approximate cost per acre for the materials used in the successful treatments is given. Some of the more promising treatments requiring no water are sulphur, $220 per acre, and kerosene, $765 per acre. Two promising treatments requiring water are lime-sulphur, $726 per acre, and Bordeaux mixture, $315 per acre. Bichloride of mercury in various combinations seems undoubtedly effective but costs $1,900 per acre for materials. Steam-pan treatments if sufficiently prolonged would be effective where usable but would probably cost at least $1,500 per acre.

PART II.—TEMPERATURE CHANGES OCCURRING IN SOIL UNDER A STEAM PAN

INTRODUCTION

In connection with the test of steam-pan treatments as a possible means of exterminating the potato-wart organism in the United States, data were secured as to the temperature changes occurring in the soil under the steam pan. If a given amount of steam heat is fatal to the potato-wart organism, a knowledge of the conditions necessary to insure that amount of heat in each type of soil to be treated and to the proper depth is essential to efficiency in using the treatment on a large scale.

Temperatures obtained in soil treated by the steam-pan method have apparently received relatively little attention. A number of investigators, including Russell and his associates (*15*, *16*, *17*, *18*) in England, and Johnson (*10*, *11*) in this country, have studied the effect of heat on soil and on plant growth, but no schedule of steam-pan temperatures was found in the literature. So far as the writers have been able to discover, the most complete schedule of temperatures obtained under a steam pan are given by Beinhart (*2*), who says, "In sandy soils, after 30 minutes of steaming, the temperatures to be expected in the upper two inches of soil directly under the pan are approximately 208° to 212° F., at 3 to 4 inches 170° to 180°, and at 6 inches 120°. Two hours after the removal of the pan the temperature at 6 inches should be about 160° F."

The work herewith reported gives a much more comprehensive series of temperatures than those of Beinhart.

APPARATUS AND METHODS

The construction of the steam pans used in the potato-wart extermination experiments is explained on pages 303 and 304.

The source of steam was a high-pressure 8-inch main which carried steam for use in the coal mines for pumping, hoisting, and other purposes. The pressure in the main was almost constant, usually varying only between 135 and 140 pounds, but often dropped appreciably for a few moments, particularly when hoisting outfits were being started. The quality of the steam varied considerably. The point at which the steam-pan supply line was attached to the main was said to be several miles from the boiler generating the steam. When rain fell on the exposed part of the pipe line there was more condensation than usual. The steam was also much poorer, i. e., contained more condensation water, when the mines were using little steam.

During the early part of the season the steam pan was located 200 feet from the large main, ¾-inch pipe being used to carry the steam almost to the pan, 20 feet of steam hose being used at the pan. Later in the season the pan was used only 75 feet from the steam line. Steam at 140 pounds pressure is not readily managed and is not always available, hence it was thought best to reduce the pressure. Beinhart and others recommended the use of steam at 100 pounds boiler pressure with a minimum of 70 pounds. In the writers' work a gauge was placed on the pipe line near the point of attachment of the steam hose and only enough steam turned on the closed line to give a reading of 90 to 95 pounds on this gauge. This method of regulating the steam was not entirely satisfactory when the steam pan was 200 feet from the main. When the pan was moved to 75 feet from the main it was found practically impossible to have the gauge pressure at 90 to 95 pounds with the line closed and then have any steam flow at all when the steam was turned on the pan. It was found desirable to take the gauge reading with the steam running wide open into the air. Under these conditions a gauge reading of between 5 and 10 pounds, nearly 10 pounds, seemed to give a flow of steam equal to that formerly obtained with the 95 pounds pressure. The observations made led to the conclusion that the flow of steam was probably different following each separate setting of the valve by gauge reading with the line closed, even when the pan was 200 feet away. In order to secure an accurate index of the steam flow a

reducing valve or "pressure-regulator" was secured. A small gauge was put on near the end of the line of pipe and the reducing valve placed between this gauge and the steam main was set so that the gauge would read 10 pounds with the line wide open. While sudden calls on the main for hoisting, or other work in the mines sometimes made the pressure drop to 7 or 8 pounds for a moment, the steam flow was practically uniform for the entire period of steaming. By this method the steam could always be regulated in a few minutes, whereas formerly it usually took a half-hour or more. A measure of the quantity of steam flowing into the steam pan is essential for comparative purposes and to secure the uniform treatment of the different pan areas supposed to receive the same treatment. Where the pressure on the main or boiler varies, or where the outlet must be regulated to keep the boiler pressure up, even an approximate estimate of the steam flow is difficult. In such cases the use of an inexpensive reducing valve will do much to insure the delivery of uniform quantities of steam in a given time.

The gardens used in our experiments are located at the southern boundary of the glacial advance, the soil being a mixture of the DeKalb and Volusia series of silt loams. The soil in these gardens was the usual conglomeration of soil, coal dust, and débris found in the gardens of coal miners in that region. As might be expected, the soil often varied considerably in different parts of the same garden and even in different parts of the same pan area.

Electrical resistance bulbs were used to obtain the temperatures in soil under the steam pan. These resistance bulbs were connected to a Wheatstone Bridge on which the dial was graduated to show temperature in degrees Centigrade instead of showing the corresponding electrical resistance. The writers can not recommend these bulbs for this work, as they get out of order too readily when held at approximately 100° C.

Soil to be given a steam-pan treatment was spaded, and if lumpy the lumps were broken up, yielding a surface layer of well-cultivated soil 8 to 9 inches deep. When temperatures were to be taken, one or more trenches were dug in this prepared soil and the leads placed in them and covered. The depth of the soil over the leads was measured by laying a board across the trench and measuring from the lower edge of the board to the top of the cap or bulb on the end of the lead. If the depth was not that desired more soil was added or some scooped out until the bulb rested at the proper level. By carefully removing the soil after the treatment was finished and remeasuring the depth to some of the bulbs it was determined that the method of placing the bulbs was accurate. In thus putting in the leads and getting them at exactly the right level it was usually necessary to add a layer of rather fine soil. In addition, the extra handling tended to break up the soil somewhat so that the soil surrounding the leads was undoubtedly finer than the main body of soil to be treated. The soil was not compacted. The leads were put in approximately parallel to the surface, so that the channels inevitably formed where the soil was in contact with them probably had little or no effect on the results obtained. Sometimes several leads were put in the same trench at different levels. When this was done care was taken to keep the bulbs from being placed one immediately over the other, even though 2 or 3 inches of soil might intervene.

When all leads were installed the steam pan was placed on the area and preliminary temperatures were taken. The preliminary temperatures sometimes varied a degree or two, especially on hot days, even though the soil was newly spaded. The surface soil would get warmed up considerably and tend to retain its heat long enough to show when the reading was made, even if it had been used to cover one of the lower leads and had later been covered by other soil. After preliminary temperatures were recorded, steam was turned on. The steam was always allowed to run wide open into the air for a few minutes to drive out any accumulated water of condensation before the steam was turned into the steam pan. Sometimes a number of gallons of water would be blown out, sometimes practically none would come out. During the time the steam was on and for an hour afterward temperatures were usually taken every five minutes. The steam pan was almost always left on for 30 minutes after steam was shut off.

Some Variable Factors

The particular part of the pan area chosen for placing the bulbs did not seem to affect the results. The temperatures obtained with two bulbs at the same depth in different parts of the same pan did not always agree, but the variation was not constant for different tests.

For a great many pan areas the percentage of moisture present in the soil was determined, but these data are not now available. Such data as are available, however, and the impression gained from observations at the time of steaming fail to indicate any particular correlation between the amount of soil moisture and the penetration of heat. In most cases steaming did not seem to greatly increase the soil moisture. Six samples from pan areas steamed for 40 minutes and having moisture percentages ranging from 23 to 33 and averaging 27 before steaming ranged from 25 to 50 per cent, averaging 32 per cent of moisture after steaming. This is an average increase of 5 per cent. If one sample showing an increase of 20 per cent (30 to 50 per cent) is omitted the average increase is only 2 per cent.

The increase in the percentage of moisture may depend on the type of soil more than on the quality of the steam. Of the six samples averaged, as indicated above, three were from the same pan area. In this case the soil was prepared as usual and three ditches scooped out for leads. In the middle ditch a single lead was installed, using the soil that had been removed from the ditch to cover the bulb and lead. In the left-hand ditch three bulbs were installed, yellow claylike subsoil being used to fill the ditch. In the right-hand ditch three leads were packed in black soil containing a large amount of fine fibrous rootlets and humus. In the normal soil the moisture increased from 25 per cent before steaming to 26 per cent after steaming; in the clay soil the moisture was 33 per cent both before and after steaming; in the black soil the moisture increased from 30 to 50 per cent. The lead in the normal soil was buried to a depth of 4 inches. It showed a maximum temperature of 44° C. in 90 minutes. The 4-inch lead in yellow subsoil showed a maximum temperature of 55° in 90 minutes. The 4-inch lead in the black soil showed a maximum temperature of 61° in 65 minutes.

Soil samples for moisture determinations were taken from 3 to 5 inches below the surface in order to get soil more nearly representing

the average. The surface soil was, of course, always damp or wet when the pan was removed, but lost much of this excess moisture within a few hours.

An attempt was made to determine the temperatures in undisturbed soil by inserting the leads in horizontal holes made by driving a rod in from a trench along one side of the soil to be treated. It did not seem possible to place the leads accurately enough in this way to get results that could be averaged without running a large number of tests. The results obtained did not seem to indicate that any marked differences in temperatures would be found between the newly spaded and the unspaded soil. If this should be found to hold true under all conditions it would obviate the necessity for cultivating the soil before steaming it.

DISCUSSION OF TEMPERATURES OBTAINED

A summary of the more important data obtained is given in Table V. A considerable number of tests are not included in this summary because the leads were placed at other depths, or because the temperatures were not taken for a long enough time, or because something went wrong with the apparatus before the series of temperature readings was complete. Hence the small number of figures averaged does not necessarily measure the real value of the data. In any event the data given represent a great advance over those hitherto available.

In Table V, temperatures obtained at 1, 4, and 7 inch depths only are considered. The data from the different pressures and lengths of steaming are arranged to show the maximum temperatures obtained the number of minutes taken to reach that temperature, the temperatures recorded at different times afterward, the last temperature taken, the length of time elapsing after the maximum temperature was reached until the final temperature was taken, the rate at which the temperature increased from the initial to the maximum temperatures (given in minutes elapsing for each degree of rise in temperature), the rate at which the temperature declined from the maximum to the last temperature recorded (given in minutes elapsing for each degree of fall in temperature), and the "maximum sustained temperature" arrived at by averaging the temperatures that will give the highest average for a half-hour period (the table showing the minimum temperature used in securing this average as well as the maximum and average temperatures). The number of sets of figures averaged for each line of figures given in the table is shown in the column at the extreme right.

The table of temperatures (Table V) has been made sufficiently complete to enable anyone to make curves showing the rise and fall of temperatures for each treatment and depth considered and showing the amount of heat received by each depth, as indicated by these temperatures and the elapsed time, over a period of three or four hours. The maximum sustained temperatures and the pressures and times necessary to attain them, with which the results of treatments given may be correlated, are perhaps the most important figures for potato-wart work. The data as a whole may be of considerable value to others, particularly perhaps to those engaged in a study of the biological changes induced by steam-pan treatments of the soil.

TABLE V.—*Temperature changes in degrees centigrade occurring in soil under a steam pan, showing the temperatures 1, 4, and 7 inches below the surface in the treated soil during and following steam-pan treatments, at different pressures and for different lengths of time. Rates of rise and fall in temperatures and maximum temperatures sustained for 30 minutes are included*

95 pounds pressure, with outlet closed,[a] 25 minutes[b]

Depth of soil covering temperature bulbs	Initial temperature of soil	Maximum temperature of soil	Time to attain maximum temperature	Temperatures after maximum had been reached					Average last temperature taken	Average time from maximum to last temperature	Average time required per degree of increase from initial to maximum temperature	Average time required per degree of decrease from maximum to last temperature	Maximum sustained temperatures maintained for one-half hour			Sets averaged
				10 min.	20 min.	30 min.	45 min.	60 min.					Min.	Max.	Aver.	
In.	°*C.*	°*C.*	*Min.*	°*C.*	°*C.*	°*C.*	°*C.*	°*C.*	°*C.*	*Min.*	*Min.*	*Min.*	°*C.*	°*C.*	°*C.*	*No.*
1	22	88	29	81	75	70	58	50	33	215	0.45	3.6	70	89	81	5
4	23	48	72	47	47	45	43	41	33	172	2.9	11.9	44	53	47	4
7	22	29	134	29	28	28	29	29	28	107	19.1	107	27	30	28	4
95 pounds pressure, with outlet closed, 40 minutes																
1	22	98	32	96	92	88	77	63	37	225	0.42	3.3	86	99	95	4
4	20	74	54	71	70	69	65	63	45	188	1.0	6.5	47	99	71	5
7	19	38	151	37	37	37	37	36	35	110	7.9	40	33	47	37	4
10 pounds pressure, with outlet open,[c] 25 minutes																
1	21	96	20	91	88	85	74	54	34	212	0.27	3.4	85	100	94	5
4	20	74	57	73	71	68	63	59	35	209	1.1	5.6	41	99	73	5
7	19	56	100	53	51	50	47	46	34	161	2.7	8	26	89	53	5
10 pounds pressure, with outlet open, 40 minutes																
1	19	99	29	98	95	90	78	63	35	209	0.36	3.3	91	99	97	13
4	17	72	62.5	68	65	63	60	57	41	187	1.1	6	38	89	68	17
7	16	38	155	38	37	37	36	32	31	131	7.0	58	23	58	37	14
10 pounds pressure, with outlet open, 75 minutes																
1	14	100	47	100	99	99	97	91	40	291	0.55	4.8	100	100	100	3
4	14	100	62	99	98	96	92	87	54	276	0.72	6	98	100	99	3
7	15	100	70	97	95	92	85	79	52	268	0.82	5.6	96	99	97	3
20 pounds pressure, with outlet open, 40 minutes																
1	14	100	15	100	99	98	97	96	39	152	0.17+	2.5	98	100	99.5	6
4	13	100	32	99	98	96	94	84	61	134	0.37−	3.4	99	100	99	9
7	13.5	100	38	98	90	85	79	75	64	119	0.44	3.4	94	98	96	6

[a] 95 pounds pressure with outlet closed means that a gauge on the steam line registered 95 pounds before the steam outlet was opened.

[b] The number of minutes during which steam was allowed to run into the steam pan is indicated. In each case the steam pan was removed 30 minutes after steam was shut off.

[c] 10 pounds pressure with outlet open means that a reducing valve was used and the valve so adjusted that a gauge on the steam line between the valve and the outlet registered 10 pounds pressure, while steam was allowed to run freely into the open air.

It has been stated that the flow of steam secured where the pressure was regulated to 85 to 95 pounds with the outlet closed appeared to be equal to that secured when the steam was regulated to about 10 pounds gauge pressure with the outlet wide open. This opinion was originally based on the appearance and sound of the flowing steam. The table shows that the temperatures obtained were similar also. The temperatures obtained with 25 minutes of steaming at 10 pounds are abnormally high for some unknown reason, the temperature at the 7-inch depth being greater than was secured at this depth with 40 minutes of steaming at the same gauge pressure. The maxima after 25 minutes of steaming show a discrepancy largely attributable to this. The maxima obtained after 40 minutes of steaming are as nearly identical as could be expected, the 1, 4, and 7 inch maxima being respectively 98° C., 74°, 38° for 95 pounds pressure closed, compared to 99°, 72°, 38° for 10 pounds pressure open. The number of minutes elapsing after steam was turned on before these maxima were reached are almost equally comparable, being 32, 54, and 151 minutes for the 95-pound pressure group and 29, 62, and 155 minutes for the 10-pound pressure group. All three depths reached a maximum of 100° where the soil was steamed 40 minutes at 20 pounds pressure, the lengths of time to attain the maximum being 15, 32, and 38 minutes. The maximum sustained temperatures for these groups are closely correlated with the maximum temperatures.

Wart spores have not been found deeper than 8 inches below the surface of the soil. It was found that steaming for 85 minutes at 90 pounds pressure did not always prevent the occurrence of wart. It is thought that this was because the method of regulating the steam supply was inaccurate, so that the failure was due to a steam flow below that intended. The temperature table shows that at 10 pounds pressure 100° C. was reached in 70 minutes at the 7-inch depth. At the rate of penetration shown, the 8-inch depth should have reached approximately 100° at the end of 85 minutes of steaming. The maximum sustained temperature at the above 7-inch depth was 96°. Hence the maximum sustained temperature at 8 inches following 85 minutes of steaming at 10 pounds pressure should be near the boiling point also. The results obtained in wart control when correlated with recorded temperatures for the treatments used indicate that a sustained temperature somewhere near 100° is necessary to kill the wart sporangia. On this basis only plots treated for 75 minutes at 10 pounds or for 40 minutes at 20 pounds could be expected to show freedom from the disease. In fact, the maximum temperatures obtained at 7 inches in the other groups show that the treatments they represent would be utterly ineffective against organisms with only a moderate tolerance of heat—unless those organisms occurred or remained viable only in the upper 2 or 3 inches of soil. Steam was used, full force (135 pounds), on one pan area in the steam garden, the temperature at 7 inches going up to 99° in 20 minutes. The temperatures fell rapidly, however, so that the "maximum sustained temperature" was an average of temperatures with a minimum of 88°. The average for one-half hour was 93°.

In studying Table V, it should be borne in mind that penetration of heat is relatively rapid as long as it is being supplied in quantity in the steam pan, but that as soon as the steam is shut off the penetration slows up. The shorter the steaming period the longer it takes

for the 7-inch depth to reach its maximum temperature. In several cases the temperature at the 7-inch depth fluctuated, seeming to indicate that for a time the soil received heat from above faster than it was transmitted to the soil below and that later the heat was transmitted much faster than received so that the temperature became lower. The only indication of such a condition in the table is at the 7-inch depth of the first set of temperatures, 95 pounds for 25 minutes. In this case the maximum reached was 29° C. Ten minutes later the temperature was still 29°, but at the end of 20 minutes and of 30 minutes it was 28°, returning to 29° at the end of 45 minutes. So far as could be determined, the fluctuations though slight were not due to errors in the readings, which were made to tenths of a degree. The data for all four of the pan areas used in making these averages show such fluctuations.

The table shows that in most cases the temperature began to fall at the 1-inch depth almost as soon as the steam was shut off. At the 4-inch level the temperature continued to rise for several minutes after steam was shut off, except where the 100° C. was reached before the steam was shut off. At the 7-inch level the temperature continued to rise for a considerable time after steam was shut off, except where a temperature of 100° had previously been reached. The temperatures at all depths usually became somewhat uniform within 4 hours after steam was turned on, regardless of whether steamed 25 or 40 minutes, the temperatures being somewhat higher than the initial temperatures.

A comparison of the temperatures obtained with 10 pounds and with 20 pounds of steam flowing for 40 minutes shows a closer approach to the 1:2 ratio than might have been expected. With 10 pounds of steam the maximum temperatures at 1 inch, 4 inches, and 7 inches averaged 52° C. higher than the initial temperature, while with 20 pounds of steam they averaged 86.5° higher. With 10 pounds of steam the maxima were reached in 29 minutes, 62.5 minutes, and 155 minutes, as compared to 15 minutes, 32 minutes, and 38 minutes at 20 pounds steam pressure. The rates of fall in temperature after the maxima were reached, in minutes per degree, were 3.3 minutes, 6.0 minutes, and 58.0 minutes at 10 pounds as compared to 2.5 minutes, 3.4 minutes, and 3.4 minutes at 20 pounds of steam.

Ten pounds of steam for 75 minutes gives approximately the same "maximum sustained temperature" as does 20 pounds of steam for 40 minutes, viz., 100°, 99°, and 97° C. as compared to 99.5°, 99°, and 96°. It will be noticed, however, that the rates of rise and fall are approximately twice as rapid in soil treated at 20 pounds. Where a high temperature for a relatively short period would be effective the higher pressure would seem desirable on account of the saving in time.

SUMMARY

During the period of steaming, heat rapidly penetrates soil under a steam pan. After steam is turned off, heat penetrates slowly. Hence the shorter the steaming period the longer the time required for the deeper soil to reach maximum temperatures. Steam-pan treatments necessary to exterminate the potato-wart organism heat the topmost 8 inches of soil to approximately 100° C.

Doubling the steam pressure almost doubles the rate of penetration of heat in the soil under the steam pan. The rate of increase

in the temperature of soil under the steam pan varied from 1° C. in 0.17 minute to 1° in 19 minutes; the rate of decrease in temperature for the periods during which data were recorded varied from 1° in 2.5 minutes to 1° in 107 minutes; the maximum temperatures reached varied from 100° to 29°; the maximum temperatures maintained for 30 minutes varied from 100° to 28°. The variations in each case depend on the pressure of the steam used, the length of the treatment, and the depth in the soil.

PART III.—THE PENETRATION OF SOIL FUNGICIDES

INTRODUCTION

Chemical soil disinfection necessarily implies contact between the sterilizing agent and the organism whose destruction is desired throughout its vertical distribution. Some chemicals, such as copper sulphate, are adsorbed or removed from water solutions before penetrating soil more than 2 or 3 inches. Other chemicals, such as bichloride of mercury, react with the soil chemicals and rapidly lose their toxic character. If the approximate penetration ability of chemicals were known, much unnecessary work would be avoided, since it would be possible to eliminate field tests of chemicals that would not penetrate the soil and to use only such strengths and quantities of other chemicals as would be reasonably sure to penetrate to a sufficient depth. Moreover, if a chemical failed to penetrate soil readily but had other merits, it might be tested by working it into the soil.

An examination of the chemical literature available and consultation with chemists failed to disclose the existence of the needed data on the soil penetration of chemicals. Simple tests for use in determining the depth of penetration of possible fungicidal chemicals were next sought.

Many fungicides are complicated compounds. The actual chemical state of the effective element in these compounds is not always known. In the case of these and of some of the simple compounds the tests in common use may be for one of the elements making up the compounds. Positive tests might then be obtained, even though the nature of the compound had been changed by reactions with the soil and its effectiveness lost. In other words, the writers did not know whether or not the tests available could be depended on for use in soil solutions. For many of the compounds with which the writers wished to experiment, no test, or at least no usable test, was available. Any test requiring prolonged individual attention would not be usable. The possession of penetration data offered such possibilities of increased efficiency in the prosecution of the soil sterilization studies that it seemed desirable to investigate the subject as thoroughly as possible, in spite of the difficulties involved.

HISTORICAL

Heretofore soil sterilization work has been largely confined to the control of seed-bed and greenhouse diseases and under specific conditions. Little data on the amount of penetration obtained are given in the reports. Gilbert (*4*), in speaking of formaldehyde sterilization of tobacco seed beds for Rhizoctonia and Thielavia root rot, recommends drenching the soil with formaldehyde, using 1 part of formaldehyde to 150 to 200 parts of water, three-fourths to

1 gallon of solution per square foot. "The solution should be put on with a watering pot with a rose and distributed as evenly as possible over the bed so as to thoroughly wet the soil to a depth of a foot." The writers' experiments, carried out with various types of soil in different moisture conditions, show that there is considerable variation in the amount of solution necessary for thorough wetting of the soil to a given depth. Hartley (*7, 8, 9*), the leading authority on damping-off diseases of forest-tree seedlings, recommends the application of 2 to 7 c. c. of concentrated sulphuric acid in 500 to 1,000 c. c. of water per square foot of seed bed. He found that the amount of acid needed varies with the kind of soil, and the quantity of water required varies with the soil moisture. Hartley and Pierce say (*8, p. 7*): "The disinfectant must be dissolved in sufficient water to permit its distribution through the soil to a depth of several inches, but within certain limits the concentration of the solution as applied does not appear to be an important factor." Most of the coniferous seed beds had sandy soils. Where the seed-bed soil was heavy and the addition of acid caused marked effervescence, indicating alkalinity, it was found that copper-sulphate solution gave better results than the sulphuric-acid application.

Several others have carried on experimental work on the control of seed-bed and greenhouse diseases by complete or partial soil sterilization.

Halstead (*6*) tried a variety of chemicals in soil treatments, but the applications of one-tenth gallon per square foot were inadequate for penetration beyond the surface few inches at most. None of the publications consulted contain more than incidental and very general statements as to penetration. There were no data available that would enable one to plan soil treatments intelligently or with even a reasonable degree of assurance that important and variable factors had been taken into account.

The present paper records the progress made in securing fundamental data on the penetration of soil fungicides. The work involved the determination of satisfactory tests for the presence of certain chemicals in treated soil and the penetration of certain chemicals in different soils under different moisture conditions when different strengths and amounts of solution were used. Two improvements were made in the method of application of a water solution by the use of a cheap chemical to protect an expensive one in the solution, and by the application of these chemicals in part of the water followed by the remainder of the water to secure a more even distribution of the toxic chemical.

APPARATUS, METHODS, AND MATERIALS

A few preliminary experiments on formaldehyde penetration were made in the field near Freeland, Pa., in 1920. Most of the experiments were carried out in the laboratory at Washington, D. C. The use of laboratory methods and equipment made it possible to carry on the work during the winter and multiplied the amount and increased the accuracy of the data it was possible to secure.

After consultation with some members of the staff of the Bureau of Soils, equipment was ordered, including soil tubes, sieves and shaker, compactors, balances, chemicals, and ordinary laboratory supplies of glassware.

The soil tubes used were practically all 2 by 15 inch glass tubes. A few brass tubes were secured, but owing to the corrosive action on them of some of the chemicals tested their use was limited. In order to have the soil evenly compacted, standard compactors were secured. The compactor used for the brass tubes has a propeller in the tube and gives the tube quarter-inch drops as it is rotated. The other compactor is of the springboard type recommended for use with glass tubes. With this compactor the tube rests on the middle of the springboard while a weight is dropped from a definite height onto the end of the springboard. After a number of tests it was decided that six drops of the weight from the top of its guide rod gave a uniform compaction with the soils used. Eight-inch sieves of different sizes and an electric sieve shaker were secured. This shaker has a semirotary and undulatory motion, with a decided jar given the sieves when revolved. A series of sieves can be used at the same time.

Soils Used

The soil used was almost entirely Manor loam from Chevy Chase, D. C., or Leonardtown silt loam from Farlee, Va. A few tests were run with potting soil from the greenhouses, but this was found to be too variable. Manor loam is a common soil type near Washington, D. C., containing 20 per cent or more of clay and having a moisture equivalent of 20.1 per cent.[9] Leonardtown silt loam, common in nearby Virginia and Maryland, contains 15 per cent or less of clay and has a moisture equivalent of 23.8 per cent. All Manor loam soil used was from a single load of soil, and the Leonardtown silt loam was all taken from an area only a few feet in diameter. These soils were naturally uniform, and each lot was made even more uniform by thorough mixing. The region about Freeland, Pa., is transitional between the Volusia and the DeKalb series of soils. Both types are silt loams with some tendency to acidity. The coal-miners' gardens in which the wart disease occurs are conglomerations of ashes, cinders, coal dust, and soil, so that soil types for the region do not necessarily indicate what may actually be found in a given garden.

Preparation of Soil Tubes

Soil to be tested in tubes was run through a screen (quarter-inch mesh) to remove any roots, stones, and large lumps. The soil for each tube was weighed. The amount of soil used in a tube depended somewhat on the character of the soil and the tests to be run. In some cases 500 grams of the loose soil filled a tube, or the amount of penetration to be expected made the use of more soil unnecessary When the soil was in such condition that a larger amount of the loose soil would go into a tube and the amount of penetration expected made it advisable, 600 grams per tube was used. In either event the soil was compacted as described above. The height of the soil columns secured was reasonably uniform. The heights of soil columns as given were taken after treatment. The number of tubes used in a set varied greatly. The penetration secured was so uniform for similar tubes that only three or six of a set of similar tubes were ordinarily tested in any one day. This made it possible to run tests

[9] Moisture equivalents furnished by John W. McLane of the Biophysical Laboratory, Bureau of Plant Industry.

of a number of different treatments at the same time for comparative purposes. To avoid confusion the tubes used in each experiment were numbered consecutively after the experiment was set up.

How Solutions were Applied

Preliminary tests showed such uniform penetration following applications by simply pouring the proper amounts of solution from a graduate onto the soil in the tubes that no other method of application was tried. In many cases the chemicals to be applied were dissolved in part of the water and then used, the remainder of the water being applied later. For convenience notations were used to indicate this, as follows:

1+0=1 gallon of solution per square foot.
1+1=½ gallon of solution per square foot +½ gallon of water.
1+3=¼ gallon of solution per square foot +¾ gallon of water.
1+7=⅛ gallon of solution per square foot +⅞ gallon of water.
(1+1)+0=½ gallon of solution per square foot +½ gallon of solution.

In other words, these figures are based on applications totaling 1 gallon per square foot, the first figure showing the number of parts of the water used in making up the chemical solution applied and the second figure showing the number of parts of water applied later. These figures are used particularly for applications of mercuric chloride, and, unless otherwise specified, indicate the application of 1 gallon of one-half of 1 per cent mercuric chloride plus 2½ per cent sodium chloride per square foot, or its equivalent.

Determination of Soil Moisture

Although only approximate soil-moisture determinations were required, they were made with considerable care. The crucibles used for soil-moisture determinations were of known weight. Weights to balance the crucible to be used were first placed on one side of the analytical balances and a 50-gram weight added. The crucible was then removed from the desiccator and put on the other scale pan with soil added to balance. After 48 hours in the electric oven at 95° C. the crucible was placed in a desiccator to cool and was then reweighed. The weight in milligrams was recorded. The per cent of moisture equals weight lost divided by the dry weight of soil. While the time the samples were kept in the oven often varied somewhat, the percentages of moisture obtained are accurate enough for our purposes.

Method of Handling Soil Samples Tested for the Presence of Chemicals

After tubes of soil treated with chemical solutions had remained in position for the desired length of time the soil was removed an inch at a time and each inch tested for the presence of the chemical applied. Before removing any soil from a tube a wax pencil was used to mark the tube off into 1-inch sections, starting from the top. The bottom inch or fraction of an inch of soil was then carefully removed with a spatula and stirred up with 50 c. c. of water in a 250 c. c. beaker. The number of the tube from which this soil section was taken and the number of inches from the top of the soil column to this particular section was marked on the beaker. The same procedure was followed until each inch of soil in the tube had been similarly treated. When the soil in the beaker had settled, the clearer liquid on the surface was decanted and filtered into test tubes. This

filtrate was then tested for the presence of the chemical with which the soil had been treated. After some experience it was often possible to estimate the depth of penetration, so that only a few tests for each tube were necessary.

The Chemical Tests Used

TEST FOR FORMALDEHYDE

The first test made was one for formaldehyde. A number of tests, particularly Schiff's, were tried without success. Schryver's test as given by Haas and Hill (*5 p. 59*) was found to give good results in general. The method is as follows:

> To 10 c. c. of the liquid to be tested add 2 c. c. of a 1 per cent solution of phenylhydrazine hydrochloride freshly made up and filtered; then add 1 c. c. of a 5 per cent solution of sodium ferricyanide, also freshly made up, and 5 c. c. of hydrochloric acid; a brilliant magenta color is produced. The test is a very delicate one and will detect quantities of formaldehyde varying from 1 part in 1,000,000 to 1 part in 100,000. Acetic aldehyde gives no color with this reagent

In preliminary work [10] with soil solutions to which known quantities of formaldehyde had been added, successful tests were obtained in dilutions as great as 1 part in 1,250,000. Yet in some cases positive tests were not obtained in dilutions greater than 1 part in 250,000. Slight differences in the weighing or measuring of chemicals, in their uniformity, or in the age of the solutions, may have been responsible for the variations. The color produced is brilliant only when there is sufficient formaldehyde present. When nearing the limit of dilution for positive tests the color produced is very faint pink. In the case of a few soil samples a deep green color was produced, completely screening the red color if such were produced. This green color was not encountered in soil except where foreign material was present. It seemed to be due to the presence of iron in certain forms.

TEST FOR MERCURY AND COPPER

Hydrogen sulphide was used as a test for mercury bichloride and copper sulphate. For convenience water was charged with hydrogen sulphide gas and some of this added to the clear filtrate obtained from the soil solution. When the amount of metal present was small the precipitate would cause only a faint browning of the solution. It had been feared that this test would be unusable owing to possible action of soil chemicals. There was no visible evidence that the hydrogen-sulphide tests were affected by soil chemicals at any time.

Manufacturers are now testing a number of mercury compounds as possible disinfectants and fungicides. Some of these compounds do not form Hg-ions in water solutions and therefore are not precipitated by H_2S. One of the manufacturing companies suggested that we ignite the soil, boil gently in acetic acid, filter, and precipitate with ammonium sulphide. The presence of iron in the soils caused an excessively heavy precipitate which obscured any mercury precipitate that may have been present. It had been suggested that these non-ionizing compounds should penetrate better than mercury bichloride. Without a usable test for their presence in soil solutions the writers have been unable to test this theory.

[10] L. O. Overholts of Pennsylvania State College, performed many of the preliminary experiments with the formaldehyde tests.

TEST FOR KEROSENE

No chemical test could be found for kerosene, nevertheless some experiments were run with it. The penetration of the kerosene was tested by three methods. The soil sample on removal from the tube was smelled, then part of it was placed on a sheet of white paper and the remainder put in water. Kerosene makes a transparent spot on paper and makes a film of oil on water; or, if present in larger amounts, forms drops of oil on the surface of the water. Repeated tests checked by several persons indicated that the odor test was shorter and perhaps more accurate than the others. The odor test was checked by the oil-film-on-water test each time. These tests may be far from accurate. In many cases the odor of the soil was changed markedly for two inches below the point which gave a characteristic kerosene odor. Whether this change in odor indicated the presence of small amounts of kerosene or of some penetrating constituent of the kerosene such as vapor or soil-water film, the writers do not know.

TEST FOR ALCOHOL

To test for the presence of alcohol a few crystals of iodine were dissolved in the filtered soil solution and a few drops of a dilute solution of sodium hydroxide were added. The test tube was then gently heated if necessary. If alcohol was present an odor of iodoform was given off, and if the amount of alcohol was not too small, yellow crystals of iodoform were precipitated.

TEST FOR QUA-SUL

The chemical test used to determine the presence of Qua-sul was a simple precipitation test. When concentrated hydrochloric acid was allowed to drop into dilute solutions of Qua-sul, free sulphur was precipitated.

TEST FOR SODIUM CHLORIDE

To test for the presence of salt in soil solutions a dilute solution of silver nitrate was used. The addition of a few drops of the silver nitrate solution to the soil solution filtrate caused the formation of a white precipitate if sodium chloride was present.

PRELIMINARY PENETRATION TESTS

PENETRATION OF FORMALDEHYDE

Almost immediately after the chemical test for the presence of formaldehyde in soil solutions was found, experiments were begun to determine the penetration of formaldehyde in soil in the experimental gardens at Freeland, Pa. Some plots 1 yard square were laid out and treated with formaldehyde at the rate of ½ gallon per square foot. Samples from near the center of these small plots were tested. All tests of soil taken from a depth greater than 4 inches were negative for most plots. Occasionally formaldehyde was found below 4 inches, in one or two cases as deep as 8 to 9 inches. A few preliminary experiments were carried out in the laboratory at Washington, D. C., with tap water to determine the approximate amounts of soil required to fill the tubes used and of water and time required

to secure percolation of the water through the tubes. Using this preliminary data as a basis, laboratory tests with formaldehyde were planned.

The first tests were made with six brass tubes in series. The tubes were filled and then compacted with the compactor for metal soil tubes. The column of soil was usually 14½ inches deep after compaction. Just enough solution was supplied to keep it at the overflow level in all tubes. The time was recorded when solution was turned on the tubes and again when percolate began to drip from the bottom of each tube. The percolate was tested for formaldehyde and gave a strong positive test in each case. Three strengths of formaldehyde were used, viz, $\frac{1}{20}$ per cent, ¼ per cent, and 1 per cent. The rate of percolation in air-dry (3 to 4 per cent moisture) Manor loam soil varied from 7.8 to 11.4 minutes per inch, averaging 9.6 minutes per inch for the 23 tubes used. The rate of percolation in potting soil was much more rapid. Six tubes of potting soil with 3.13 per cent of moisture showed percolation at the rate of 4 minutes per inch. Six tubes with 11.3 per cent moisture showed percolation at the rate of 3 minutes per inch.

Penetration of Copper Sulphate

In tests with 1 per cent copper sulphate solution in potting soil (6 tubes) containing 7.6 per cent of moisture the average rate of percolation was 4 minutes per inch. With a 2 per cent solution of copper sulphate, using Manor loam (6 tubes) containing 6.3 per cent of moisture, the rate of percolation averaged 11 minutes per inch. No positive test for copper was secured in the percolate from any of these tubes.

Penetration of Alcohol

Alcohol, 47 per cent, in one test with six tubes of potting soil containing 4.45 per cent moisture, showed percolation at the rate of 6.5 to 9.5, averaging 8 minutes per inch. The percolate gave positive tests for alcohol in each case.

Penetration of Qua-sul

Qua-sul, a proprietary sulphur compound, was tried at 1 per cent strength on six tubes of potting soil with 8.7 per cent moisture. Percolation was at the rate of 3.8 to 8.8, averaging 5.3+, minutes per inch. No sulphur could be precipitated in the percolate. The potting soil used was taken from the mixing bench.

If variations in the make-up of the soil in different tubes was responsible for the wide variations in rate of percolation, these variations in composition did not show in a casual examination of the soil.

Amount of Solution Required for Percolation

The amount of solution required to percolate through the tubes of soil was determined from the increase in weight. The tubes were weighed dry and again after the percolate started to drip, but without allowing the soil to drain. The increased weight in grams was assumed to equal the number of cubic centimeters of water held.

The maximum amount of solution held by any one of 30 tubes of potting soil was 300 c. c., the minimum 227 c. c., the average 258 c. c. The maximum amount of solution held by any one of 17 tubes

of Manor loam soil was 333 c. c., the minimum 294 c. c., the average 323 c. c. These figures show far less variation than the rates of percolation. Most sets of six tubes varied little within the set—in one case only 6 c. c. Most sets were of air-dry soil. As the tubes are 2 inches in diameter, applications of 258 c. c. per tube would be equal to approximately 3 gallons per square foot to secure percolation through 13½ inches of potting soil and of 323 c. c. per tube would be approximately 4 gallons per square foot to secure percolation through 14½ inches of Manor loam soil. At the time these laboratory tests were started the field applications had not exceeded 2 quarts of formaldehyde solution per square foot, and tests of field-treated soil had indicated that such applications could not be depended on to penetrate more than 4 or 5 inches. A few tubes were set up with soils to which definite amounts of water had been added, and formaldehyde solution was then added to each tube at the rate of 2 quarts per square foot. The results were exceedingly erratic. To other sets of tubes applications were made at the rate of 1 gallon per square foot. Of 9 tubes set up with Manor loam put through an 8-mesh-to-the-inch sieve, 2 tubes showed penetration 9 to 10 inches, 5 tubes 8 to 9 inches, and 2 tubes 7 to 8 inches at the end of 1 day. These results indicated that it would be necessary to use applications of 1 gallon or more per square foot in order to secure the 8 inches of penetration required to reach all wart spores in the soil.

Penetration of Formaldehyde Compared with that of Mercuric Chloride

The experiments with formaldehyde indicated that the penetration of formaldehyde in water solutions practically coincided with the penetration of the water of the solution. The test for formaldehyde while sensitive and accurate and relatively simple is far more tedious than the simple precipitation test with H_2S for mercury. Preliminary determinations were made to see if the H_2S precipitate test could be successfully used in soil solutions. No positive results were obtained with this test at any depth in either potting soil or Manor loam treated with 1 gallon per square foot of 1 to 1,000 mercuric chloride solution. Three tubes of potting soil were then given applications of 1 per cent mercuric chloride at the rate of 3 gallons per square foot. A faint mercury test was obtained in the percolate from each tube. These tests indicated rapid removal of the mercury from the solution as it passed through the soil. To retard this removal of the mercury, sodium chloride was added to the mercuric chloride solution at the rate of 5 parts of salt to 1 part of mercuric chloride. Salt was first added to mercury in our field treatments because Hartley (7) reported that the combination was particularly severe on green plants when used in soil treatments. It was thought possible it would be equally severe in its action on the wart fungus. In studying the penetration of mercuric chloride it seemed wise to test the penetration of the solution actually used in the field experiments. The penetration was found to be very much better when salt was added to the solution. Manor loam with a moisture of 9.5 per cent and potting soil with a moisture of 6.8 per cent were used for parallel sets of tubes treated with 1 per cent formaldehyde and with 2 per cent mercuric chloride plus 10 per cent sodium chloride, at the rate of 200 c. c. per tube (nearly

2½ gallons per square foot). Six glass tubes were used for each of the four sets and the soil columns were from 9 to 10½ inches deep. Strong positive tests for formaldehyde or for bichloride of mercury were obtained in the percolate of all tubes. Percolation of the formaldehyde solution was more rapid than that of the mercuric chloride solution. In Manor loam the formalin solution percolated in 29 to 35 minutes, averaging 32 minutes, while mercuric chloride percolated in 38 to 54 minutes, averaging 49 minutes. In potting soil the formalin solution percolated in 4 to 27 minutes, averaging 8 minutes, while mercuric chloride percolated in 12 to 14 minutes, averaging 13 minutes.

Manor loam soil containing 8.2 per cent of moisture, and potting soil containing 6.8 per cent of moisture were used in two paralell sets of tubes treated with 2 per cent mercuric chloride and with 2 per cent mercuric chloride plus 10 per cent sodium chloride at the rate of 2 gallons per square foot. Three hours after the tubes were treated the percolate from the 12 potting-soil tubes gave positive tests for mercury. In three days the Manor loam tubes were tested and positive tests secured in the bottom inch in each case. There was no percolate from the Manor loam tubes. The height of the soil columns varied from 9¼ to 10½ inches at the time of testing.

Penetration of Mercuric Chloride

Standard application of mercuric chloride

The foregoing experiments showed that mercuric chloride with its simple and rapid H_2S test might be successfully used in penetration studies. The strength of solution was modified on the basis of further experiments, some of which are recorded below. One gallon of ½ per cent $HgCl_2$ + 2½ per cent NaCl solution, or its equivalent, per square foot was adopted as the standard application. In a comparative test of applications of 1 gallon of ½ per cent mercuric chloride + 2½ per cent sodium chloride versus ¼ per cent mercuric chloride + 1¼ per cent sodium chloride, tubes tested at the end of two days and others at the end of three days showed 9 to 10 inches penetration for the stronger solution, and 8 to 9 inches penetration for the weaker solution. The soil in these tubes contained 15.1 per cent moisture. That mercuric chloride alone in solution does not give satisfactory penetration at similar strengths and applications is indicated by a test on soil containing 11.9 per cent of moisture. One gallon of 1 per cent mercuric chloride solution showed 6 to 7 inches penetration in one day and the same in two days, and ½ per cent solution showed 4+ inches penetration in one day and 5 to 6 inches in two days.

The above tests were on Manor loam. Later Leonardtown silt loam containing 6.23 per cent moisture was treated with 2 quarts of 1 per cent $HgCl_2$ + 2 quarts of water. At the end of 2 days 5 tubes showed 7 inches penetration, in 8 days 4+ inches, and in 11 days 5 to 6 inches. This would seem to indicate the gradual removal of the bichloride of mercury after penetration.

Reducing the proportion of salt from five times to twice the amount of mercuric chloride reduced penetration (Table VI). Applying the solution in two parts did not give as good results as applying the chemicals in half the water and applying the other half subsequently.

TABLE VI.—*Effect of reducing proportion of NaCl to $HgCl_2$ in 1+1 and (1+1)+0 applications*

Method of application	Solutions applied		Penetration	
	First application	Second application	In 3 days	In 4 days
			Inches	*Inches*
1+1 [a]	1 per cent $HgCl_2$+5 per cent NaCl, 2 quarts per square foot.	Water, 2 quarts per square foot.	8+	9
(1+1)+0	½ per cent $HgCl_2$+2½ per cent NaCl 2 quarts per square foot.	½ per cent $HgCl_2$+2½ per cent NaCl, 2 quarts per square foot.	7 to 8	7 to 8
1+1	1 per cent $HgCl_2$+2 per cent NaCl, 2 quarts per square foot.	Water, 2 quarts per square foot.	7 to 8	8 to 9
(1+1)+0	½ per cent $HgCl_2$+1 per cent NaCl, 2 quarts per square foot.	½ per cent $HgCl_2$+NaCl, 2 quarts per square foot.	7 to 8	6 to 7

[a] See page 332, "How solutions were applied" for further explanation of these symbols. Each figure is the average for 3 tubes. Soil used, Manor loam containing 10.5 per cent of moisture.

STANDARD TREATMENT

On the basis of these and other preliminary experiments 1 gallon per square foot was adopted as the standard application and the amount of chemicals required to make 1 gallon of ½ per cent $HgCl_2$+2½ per cent NaCl solution was adopted as the standard quantity of chemical to be applied per square foot. It was not deemed wise to spend the time necessary to fix the amount of chemicals more accurately, especially in view of the fact that it seemed certain to vary with different soils.

PENETRATION OF $HgCl_2$ IN POTTING SOIL

Penetration of mercuric chloride in potting soil was so erratic that experiments with potting soil were discontinued. Using 1 gallon of solution (or of solution plus water) the penetrations, as determined by tests with H_2S, were as follows: In air dry (2 per cent moisture) potting soil penetration varied from none at the end of five days to 7 to 8 inches at the end of one day (18 tubes); in soil containing 11.4 to 14.8 per cent moisture the maximum penetration secured was 2 to 3 inches (36 tubes); in soil containing 17.6 to 17.8 per cent moisture penetration varied from 7 to 8 inches to 10 to 11 inches (36 tubes). In 13 of the tubes filled with soil containing 11.4 or 14.8 per cent moisture no positive test was obtained at any depth. Two of these were tested at the end of one day. Evidently the mercuric chloride was removed from solution or else its character changed very soon after application. Whether the differences in results were due to varying amounts or types of fertilizers in the soil used or to other causes was not determined.

PENETRATION OF $HgCl_2$+NaCl SOLUTIONS IN MANOR LOAM AS SHOWN BY H_2S TESTS

As shown in Tables VII and VIII, the penetration of mercuric chloride in solutions [11] containing sodium chloride and applied at the rate of 1 gallon per square foot was determined in soil containing different percentages of moisture. In many of the experiments the

[11] Unless otherwise stated standard treatments were given.

chemicals were applied in part of the water and the remainder of the water was applied subsequently. In soil containing 4 per cent or less of moisture the average penetration was about 7 inches; in soil containing 10 to 16 per cent or 20 to 23 per cent of moisture the penetration averaged 8 inches; while in soil containing 17 to 19 per cent the penetration averaged somewhat more than 8 inches. The depth of penetration seems to have been about the same whether the chemicals were applied in all or in part of the water.

TABLE VII.—*Penetration of mercury in mercuric chloride plus sodium chloride solutions in Manor loam, as shown by hydrogen sulphide. Average penetration (inches) at different percentages of soil moisture of applications totaling 1 gallon per square foot*

In soil containing 4 per cent moisture

Proportion of solution + water	1 day	2 days	3 days	4 days	5 days	6 days	7 days	9 days	Number of tubes
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	
1+0 [a]	7 to 8		7−		7−				9
1+1	7	7+	7+	8−	8−				48
1+3		6+	7+	7+	7+			8 to 9	27
1+7			7		7+				6

In soil containing 10 to 16 per cent moisture

1+0		8	8+	7 to 8			8 to 9		18
1+1		8−	8−	8+			8 to 9		30
1+3		7+	8−	6 to 7		7 to 8			18
1+7		7+	8−	8 to 9		8+			12

In soil containing 17 to 19 per cent moisture

1+0	8−	9	9 to 10	9 to 10			9 to 10		24
1+1	8 to 9	8+	8+	9	9 to 10	9	9 to 10	9 to 10	40
1+3	9−	9	8+	8+	8+	9−	8+	9 to 10	70
1+7	8−	9−		8+	8 to 9	8 to 9	8+		36

In soil containing 20 to 23 per cent moisture

1+0		6+	7 to 8	7−	8−				12
1+1		7	7 to 8	7 to 8		7 to 8		8 to 9	14
1+3		8−	9 to 10	8+		9			12
1+7		7−	8−	8 to 9		8 to 9			12

SUMMARY [b]

Per cent moisture	1 day	2 days	3 days	4 days	5 days	6 days	7 days	9 days	Number of tubes
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	
4	7−	7−	7+	8−	7+			8 to 9	90
10 to 16		8−	8−	8−	9−	8−	8 to 9		78
17 to 19	8−	9−	8+	8+	8+	8+	9−	9 to 10	170
20 to 23		7−	8−	8−	8−	8−		8 to 9	50
Total									388

[a] 1+0=1 gallon ½ per cent $HgCl_2$+2½ per cent NaCl per square foot.
1+1=½ gallon 1 per cent $HgCl_2$+5 per cent NaCl+½ gallon water per square foot.
1+3=1 quart 2 per cent $HgCl_2$+10 per cent NaCl+3 quarts water per square foot.
1+7=1 pint 4 per cent $HgCl_2$+20 per cent NaCl+7 pints water per square foot.
See p. 332, "How solutions were applied."

[b] This combines the data contained under 4, 10 to 16, 17 to 19, and 20 to 23 per cent moisture.

TABLE VIII.—*Number of tubes giving positive tests for mercury with the hydrogen sulphide test, at the various depths on each day after application to Manor loam of 1 gallon per square foot of mercuric chloride plus sodium chloride solutions, or of 1 gallon of solution plus water* [a]

In soil containing 4.3 per cent or less moisture

Depth of penetration	1 day	2 days	3 days	4 days	5 days	6 days	7 days	9 days	Total number of tubes
Inches:	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*	
6 to 7	3	2	3		1				9
7 to 8	6	9	23	3	13				54
8 to 9		1	7	6	9			3	26
9 to 10					1				1

In soil containing 10 to 16 per cent moisture

6 to 7	3			3					6
7 to 8	3	21	15	6		3			48
8 to 9		13	19	15	8	2	6		63
9 to 10		5	15	3	4	1			28

In soil containing 17 to 19 per cent moisture

7 to 8	6	1	3	4		2			16
8 to 9	13	9	6	13	8	11	15		75
9 to 10	5	9	11	18	7	10	15	2	77
10 to 11		2	1	1		1			5

In soil containing 20 to 23 per cent moisture

6 to 7		5							5
7 to 8		4	7	5		4			20
8 to 9		2	2	5		6		3	18
9 to 10		1	3	1		2		1	8
Total									459

[a] This table includes the tubes from some sets used in special experiments which were not included in Table VI. The amounts of chemicals used per square foot in these special experiments varied from the equivalent of those required for one gallon of ½ per cent $HgCl_2$+2½ per cent NaCl, the standard application. Since no increased penetration seemed to result from such increased amounts of chemicals it seemed permissible to include them here.

The data are arranged to show the number of days elapsing from the time of application of the solution until the penetration tests were run. It will be noted that the maximum penetration was usually not obtained in less than three days. As previously stated penetration was determined by testing soil an inch at a time. A positive test in the soil sample from the 8 to 9 inch depth was considered 8 inches in tabulating, even though the actual penetration may have been nearer 9 inches. Hence one may find an average penetration of 8 inches shown in the tables, whereas the penetration for the tubes included averaged more than 8 inches. In two tubes in which the penetration was 8¼ and 8½ inches, positive tests would be secured in the 8 to 9 inch soil section and both tubes would be listed as showing 8 inches of penetration. A tube in which penetration was barely 8 inches would give a positive test in the 7 to 8 inch soil section and hence would be listed as showing 7 inches of penetration. The average of these three tubes, 8+8+7, would appear as 8− inches in the table instead of 8¼ inches. Although this may seem inexact it must be borne in mind that for absolute extermina-

tion of a soil organism the minimum penetration is the important figure and also that owing to the time and labor involved tests were necessarily made only by inches.

In Table VIII the data are arranged to show the number of tubes giving positive tests at various depths each day. The daily increase in the proportion of positive tests at lower depths is very marked in the dry soil. The proportion of positive tests at 6 to 7 inches and at 7 to 8 inches is 1 : 2 at the end of the first day, becomes 1 : 4 in two days, 1 : 8 in three days, and 1 : 13 at the end of five days. At the same time there is a marked increase in the number of positive tests at 8 to 9 inches. The proportions do not change so widely and regularly at the other soil moistures, but the change is quite marked.

Penetration of Mercury in $HgCl_2$+NaCl Solutions in Leonardtown Silt Loam

Penetration of $HgCl_2$ + NaCl solutions in Leonardtown silt loam was studied in the same way as penetration in Manor loam and similar tabulations of the data obtained are given in Tables IX and X. As in the Manor loam, the penetration in the drier soil averaged about 7 inches, this depth only being exceeded by part of the tubes where the application was made 1+1, that is, the chemicals were applied in half the water and an equal amount of water was applied later. The penetration in soil containing 8 to 15 per cent of moisture averaged 8 inches or a little more, only slightly less than the average penetration in soil containing 15 to 21 per cent of moisture. Penetration in soil containing 43 per cent of moisture was extremely rapid as well as approximately 3 inches deeper than in soil containing 21 per cent or less of moisture. Of the 34 tubes, 21 gave positive tests for mercury in the solution that percolated through the tubes, and 7 gave positive tests in the bottom inch of soil. The figures given therefore do not represent the absolute maximum of penetration for such wet soil. As in Manor loam, the maximum penetration was usually not obtained in less than three days, and depth of penetration did not seem to be particularly affected by applying the chemicals in part of the water and then applying the remainder of the water.

In Table X the penetration data are arranged to show the number of tubes giving positive tests at each depth on successive days after the solutions were applied. The successive increases in the proportion of positive tests at lower depths are not so marked as in the Manor loam, although it is evident, particularly in the soil containing 8 to 15 per cent of moisture.

Discussion of Penetration of $HgCl_2$+NaCl Solutions

Effect of Soil Type and Soil Moisture

A comparison of the data in Tables VII and IX shows slightly better penetration in air-dry Manor loam than in air-dry Leonardtown silt loam. In Manor loam containing 10 to 16 per cent moisture the penetration was practically the same as in Leonardtown silt loam containing 8 to 15 per cent moisture. In Manor loam penetration was better in soil containing 17 to 19 per cent moisture than in soil containing 20 to 23 per cent moisture. In Leonardtown silt loam

TABLE IX.—*Penetration of mercury in mercuric chloride +sodium chloride solutions in Leonardtown silt loam. Average penetration (inches) at different percentages of soil moisture of applications totaling 1 gallon per square foot*

In soil containing 1.3 to 4.3 per cent moisture

Proportions of solution and water	1 day	2 days	3 days	4 days	5 days	6 days	7 days	12 days	Number of tubes
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	
1+0[a]		6−			6+				6
1+1		7−	7+	8	7−		8 to 9		33
1+3			7−		6 to 7				6
1+7			5 to 6		6+				6

In soil containing 8 to 15 per cent moisture

1+0	7−	7+	8				8−		12
1+1	8	8−	7 to 8	8+	8+	8 to 9	8 to 9		42
1+3	8 to 9	8−		8+	8 to 9	8 to 9	8 to 9		24
1+7	8−	8−		8+	8−	8+	8 to 9		24

In soil containing 15 to 21 per cent moisture

1+0		8 to 9	8 to 9	8+		8 to 9			12
1+1		9−	9	9	9−	9−		8−	75
1+3		9−	8 to 9	8 to 9		8+			12
1+7		8 to 9	8 to 9	8−		8+			12

In soil containing 43 per cent moisture

1+1	10+	11 to 12		11−	10−				11
1+3	11−	11 to 12		12−	12−				12
1+7	12 to 13	11+		12−	12 to 13				11

SUMMARY[b]

Moisture in soil (per cent)	1 day	2 days	3 days	4 days	5 days	6 days	7 days	12 days	Number of tubes
1 to 4		6+	7−	8−	6+		8		51
8 to 15	7+	8−	7+	8+	8+	8+	8−		102
15 to 21		8+	8+	8+	9−	8+	8−	8−	111
43	11	11+		11+	11+				34
Total									298

[a] See "How solutions were applied," p. 332, and footnote of Table VII.
[b] This combines the data contained under 1.3 to 4.3, 8 to 15, 15 to 21, and 43 per cent moisture.

there did not seem to be any real change in the depth of penetration as the percentage of moisture increased from 15 to 21 per cent. In the case of the Manor loam the soil containing 20 to 23 per cent had reached or exceeded the moisture equivalent percentage (20.1 per cent) for that soil, whereas in the Leonardtown silt loam the moisture equivalent is 23.8 per cent, or somewhat higher than the maximum moisture in the 15 to 21 per cent group of tubes. It has been suggested that there may be some correlation between the moisture equivalent and the penetration obtained. This has not been investigated. The only tests of Leonardtown silt loam containing more than the moisture equivalent were the tubes containing 43 per cent of moisture. These tubes were set in 8 inches of water for two days and then drained a short time (part of one day) before being treated.

TABLE X.—*Number of tubes giving positive tests for mercury at the various depths on each day after application to Leonardtown silt loam of 1 gallon per square foot of ½ per cent mercuric chloride + 2½ per cent sodium chloride solution, or its equivalent in chemical solutions plus water*

Depth of penetration	1 day	2 days	3 days	4 days	5 days	6 days	7 days	12 days	Total
Inches	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*	*Tubes*
In soil containing 4 per cent or less of moisture									
4 to 5		1							1
5 to 6			3		1				4
6 to 7		3	3		9				15
7 to 8		6	8	3	5				22
8 to 9			3	3			3		9
In soil containing 8 to 15 per cent moisture									
6 to 7	1	3							4
7 to 8	6	7	3	1	1	2	1		21
8 to 9	8	16		18	10	5	11		66
9 to 10		1		5	1	1			8
10 to 11						1			1
In soil containing 15 to 21 per cent moisture									
7 to 8				1		1	1	2	5
8 to 9		12	15	16	4	9	2	7	65
9 to 10		9	6	10	8	8			41
In soil containing 43 per cent moisture									
9 to 10					1				1
10 to 11	3			2					5
11 to 12	3	7		3	3				16
12 to 13	3	1		4	4				12
Total									298

As shown in Tables IX and X, exceptionally good penetration was obtained in these tubes. In general the depth of penetration increases as the percentage of moisture increases, though not regularly. Differences in penetration are not always shown by the penetration figures as taken. A set of tubes in which actual penetration was 7¼ inches is listed as 7 to 8 inches and in these summaries is counted as 7 inches, just as another set in which actual penetration may be 7⅞ inches. The actual depth of penetration in two sets of tubes may be the same, and at the same time strong tests may be obtained for one set of tubes and very weak tests for the other set. Difference in the strength of tests was noted, particularly in studying the penetration where chemicals were applied in part of the water and the remainder of the water added later.

PENETRATION WHERE ALL CHEMICALS WERE APPLIED IN PART OF THE WATER

Tables VII and IX indicate that penetration into the soil was about equally good whether all of the water was used in making up the chemical solution or whether only part was used and the rest applied later.[12] On the whole the figures indicate slightly better

[12] The effect of the amount of time elapsing between application of the chemical solution and application of the water is discussed later.

penetration where part of the water was applied later. The chemical tests emphasized this difference. Where all of the water was applied in the chemical solution, the test for mercury was often very faint in samples from near the point of maximum penetration. The strength of the test was apt to be variable, not only between different tubes but within a tube. When part of the water was withheld and applied after the application of the chemical solution, stronger and more uniform tests were obtained at points nearing the maximum depth of penetration, and the tests from different parts of the same tube were more nearly uniform.

What proportion of the water could be applied as such is not known. In our experiments three proportions were tried: 1+1, or equal parts of solution and water; 1+3, or one part solution to three parts of water; and 1+7, or one part solution to seven parts of water. As shown by Tables VII and IX, no one of these proportions is superior to the others in every case. In practically every case all three were equal to or superior to the 1+0 applications in the depth of penetration obtained. In applying solutions and water to the tubes the liquid was simply poured from the graduate. In some cases, especially where the amount of solution was small, it is quite possible most of the solution was applied to one side of the tube and that the water was poured on the other side of the tube. This would tend to reduce the amount of chemical forced down the tube by the water. So far as the writers are aware, the limiting factors in reducing the amount of water used in making up the solution would be the amount necessary to put the chemicals into solution readily and the amount of solution necessary to insure even distribution over the soil surface.

Penetration of Strong Solutions of $HgCl_2$+NaCl when Immediately Followed by an Application of Water and when Followed Later by an Application of Water

As previously shown, the depth of penetration of mercury in $HgCl_2$+NaCl solutions is about the same whether all the water is used in making up the solution or part of it is withheld and applied immediately after the application of the chemical solution, but the latter method gives a more even distribution of the mercury in the soil. This latter method offers another advantage in that no special apparatus is required in applying water while special apparatus and special care are required and therefore additional expenses incurred, in using mercuric chloride, on account of its corrosive action. Sometimes rain might supply at least a part of the water. Whether the water is applied with a hose or falls as rain it is important to know whether this water application must follow the chemical solution immediately or whether the water may be applied at some later and perhaps more convenient time. That final penetration of the mercury is not adversely affected by delaying the application of the water is indicated by the results given in Table XI, summarizing the penetration data from four sets of tubes. In every case the maximum penetration was the same for treatments differing only in the amount of time elapsing between the application of the first and second parts of the treatment. While these experiments gave favorable penetration with delayed applications, it is conceivable that a soil might react so rapidly with the bichloride of mercury that the delay would result in a serious reduction in the strength of the solution.

TABLE XI.—*Effect of delaying second part of applications made in two parts, 1+1, 1+3, 1+7, (1+1)+0*

Method of application	Time elapsing between two parts of application	Depth of penetration in inches: Time after application of first part until test was made: 21 hours	2 days	3 days	4 days	5 days	6 days	7 days	Soil moisture
		Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Per cent*
1+1[a]	10 to 15 minutes	7 to 8	8 to 9	[b]8+	8 to 9				14 to 16
1+1	17 hours	6 to 7	7 to 8	8−	8 to 9				14 to 16
1+0	Only one application		7 to 8	7+	7 to 8			8 to 9	10
1+1	17 hours		7+	7+	8 to 9			8 to 9	10
1+1	10 to 15 minutes		8−			9−			10
1+1	18 hours		8+			9−			10
(1+1)+0	10 to 15 minutes		8−			8 to 9			10
(1+1)+0	18 hours		7 to 8			8 to 9			10
1+3	10 to 15 minutes				9−	8+	9−	8+	18.6
1+3	20 hours				8−	8 to 9	9−	8 to 9	18.6
1+7	10 to 15 minutes				8 to 9	8 to 9	8+	8+	18.6
1+7	20 hours				8 to 9	8 to 9	8+	8 to 9	18.6

[a] For explanation of symbols, see "How solutions were applied," p. 332, and footnote of Table VII.
[b] Three tubes of each lot were tested each day. Where all three tubes gave positive tests (H_2S precipitation method) to the same depth, that depth is indicated by 7 to 8, 8 to 9, etc.; when the depth varied, plus or minus signs are used, e. g., 8+=2 tubes gave positive tests at 8 to 9 inches and the third at 9 to 10 inches; 8−=2 tubes gave positive tests at 8 to 9 inches, and the third at 7 to 8 inches. Soil, Manor loam.

RESULTS OBTAINED FROM EXPERIMENTS COVERING SPECIAL PHASES OF SOIL PENETRATION BY CHEMICALS, PARTICULARLY BY MERCURY IN $HgCl_2$+Na Cl SOLUTIONS

In addition to the foregoing experiments, the data from which are included in Tables VI to XI, a number of experiments were conducted to secure data on other phases of the problem of chemical penetration in soil. These include experiments to determine: (1) The effect of the size of soil particles on the depth of penetration; (2) the effect of a high water table on the depth of penetration; (3) the amount of lateral penetration of mercury in the soil; (4) the upward penetration of mercury in soil; (5) the depth of penetration in tubes which had been allowed to stand for three months after being set up and before being treated; (6) the effect of passage through soil on the toxicity of a solution of $HgCl_2+NaCl$; and (7) the relation of depth of penetration to the amount of liquid applied.

EFFECT OF SIZE OF SOIL PARTICLES ON THE PENETRATION AND PERCOLATION IN THEM OF CHEMICAL SOLUTIONS

It is well known that different soils are composed of different proportions of various sizes of soil particles. In gravelly soils, composed of very coarse particles, water penetrates with great ease and rapidity. In clay soils, composed of very fine particles, water penetrates with difficulty and very slowly. Samples of Manor loam and of Leonardtown silt loam were sieved and tests made to determine penetration in the various sizes of soil particles obtained. The soil samples were air dried, rolled fine and sieved with an electric sieve shaker. The sieves used had 20, 40, 60, 80, 100, 150, and 200 meshes per inch. It was found that a considerable proportion of the soil went through all of these sieves, that practically all soil passing through the 60-mesh sieve passed also through the 80-mesh sieve so that very little soil of this texture was available for the experiments, and that the proportion of soil held by the other sieves varied considerably.

The chemical solutions used in testing penetration in the sifted soil were $HgCl_2 + NaCl$ (1+1) and 1 per cent formaldehyde (commercial solution). The penetration data secured in these sieved soils is tabulated in Table XII. As shown in this table penetration was better in the coarse than in the fine soil. However it does not seem likely, from the results secured, that variations in the proportions of the various sizes of particles would be sufficient to cause any important variations in the depth of penetration in soils of the same general type.

TABLE XII.—*Effect of size of soil particles on penetration*

Soil	Manor loam, air dry		Leonardtown silt loam, 4.9 per cent moisture
	Mercuric chloride + sodium chloride 1+1 [a] penetration in 3 days	1 per cent formaldehyde 1+0 [a] penetration in 17 to 18½ hours	Mercuric chloride + sodium chloride 1+1 [a] penetration in 3 days
	Inches	*Inches*	*Inches*
Whole	6 to 7 [b] (2) 7 to 8 [b] (1)		7 to 8 (3).
20 to 40 [c]	8 to 9 (9)	8 to 9 (6)	7 to 8 (3).
40 to 60	6 to 7 (6) 7 to 8 (3)	7 to 8 (3) 8 to 9 (3)	7 to 8 (3).
60 to 80 [d]			6 (1) to bottom of column.
80 to 100	6 to 7 [d] (2)	6 to 7 (6)	6 to 7 (1) to bottom of column.
100 to 150	6 to 7 (1)	6 to 7 (6)	
100 to pan [e]			4 to 5 (3).
150 to 200	6 to 7 (4)	5 to 6 (2), 6 to 7 (3)	
200 to pan	5 to 6 (4)	5 to 6 (1), 6 to 7 (5)	
Leonardtown silt loam whole.	6 to 7 (1), 7 to 8 (2)		

[a] 1+1=½ gallon 1 per cent $HgCl_2$+5 per cent NaCl+½ gallon water per square foot. 1+0=1 gallon 1 per cent formaldehyde (commercial) per square foot.

[b] Numbers inclosed in parentheses indicate the number of tubes showing the depth of penetration indicated.

[c] The first figure gives the number of meshes per inch in the sieve the soil passed through and the second figure is the number of meshes per inch in the sieve holding the soil.

[d] The sifted soil was not equally divided among the various sizes. Very little was held in the 80-mesh sieve particularly, and not enough in some other sieves to run a full series of tests.

[e] The 150 and 200 mesh sieves were not available when the Leonardtown silt loam was sifted.

That the rate of penetration of a chemical solution in soil particles of different sizes may vary considerably is indicated by an experiment with formaldehyde in Manor loam. The six brass tubes were set up and 700 grams of sieved soil compacted in each tube. A 0.05 per cent solution of commercial formaldehyde was supplied to these tubes until percolate dripped from the outlet of all tubes. The percolate gave a strong positive test for formaldehyde in every case. The mesh of the sieves is given as in Table XII, together with the height of the soil column through which the solution passed and the time elapsing after the solution was turned on until percolate appeared. The weight of solution held at the end of the test by each tube of soil is also given. The results of the experiment are shown in Table XIII. The figures in the table show that the volume of the soil did not vary greatly and bore no relation to the rate of penetration. The penetration in the smallest particles was so slow that inspections were reduced to one per hour and a small amount of percolate had accumulated in the beaker under the drip when the inspection was made at the end of the tenth hour. The weight of the solution held by the 100 to 150 tube was exactly twice that held by the 20 to 40 tube. This ratio did not hold for the rate of penetration given above nor for the depth of penetration of one gallon of solution as given in Table XII.

TABLE XIII.—*Percolation of 0.05 per cent formaldehyde solution in soil particles of different sizes*

Soil	Height of soil column	Time to percolate	Water held by soil [a]
	Inches		*Grams*
20 to 40 [b]	13	16 minutes	264
40 to 60	14½	do	436
80 to 100	15¾	32 minutes	506
100 to 150	15¾	2 hours, 1 minute	528
150 to 200	14¾	2 hours, 55 minutes	407
200 to pan	14¾	9 to 10 hours	453

[a] The last column shows the gain in weight during treatment.
[b] Sieves as in Table XII. Soil=Manor loam, air dry, 700 grams per tube. A strong positive test for formaldehyde was obtained in the percolate from each tube.

EFFECT OF A HIGH-WATER TABLE ON THE PENETRATION OF MERCURY IN THE SOIL

The influence of a high-water table on the penetration of mercury in $HgCl_2 + NaCl$ solutions was tested by setting tubes of soil in beakers and adding water to the beakers. The results of these tests as shown in Table XIV were very erratic. The first set of tubes was set up and the application made to the tops of the tubes about 20 minutes after the water had been added to the beakers, and the visible water line showed that the water had risen 5 inches in the tubes. The penetration was about the same as in the tubes treated in the usual manner. The second set of tubes was set up and the chemical solution and water applied to the soil tubes almost immediately after the water had been added to the beakers. In this case the penetration was not nearly so great as in tubes treated in the usual way. The beakers were removed from half of the high-water table tubes at the end of one day to permit drainage. Penetration in these tubes then improved so that a week after the treatments were given they nearly equaled the tubes treated in the ordinary manner. It was thought that the poor penetration of the high-water table tubes in this set was due to the fact that the chemical solution only penetrated far enough to meet the upcoming flow of water. If the water is added to the beakers beforehand the upward flow will become nearly stationary in a few minutes in damp soil, the flow being slower in dry soil. With the glass tubes used the rise of the water line was easily observed.

A third set of tubes was prepared and water added to the beakers and allowed to stand overnight. The soil, being dry, had taken up practically all of the water so that more was added. In this case the tubes treated regularly did not show as good penetration as those with the high-water table. Removal of the beakers did not seem to improve penetration in this set.

The results of the fourth experiment confirmed those already obtained. The tubes receiving applications some time after water was added to the beakers showed good penetration, and tubes receiving applications at the same time water was added to the beakers showed poor penetration. An additional lot of tubes receiving applications before water was added to the beakers showed poor penetration. The results of these experiments indicate that a high water table, if stable or receding, would not prevent adequate penetration. A rising water table would be expected to interfere seriously with penetration.

TABLE XIV.—*Effect of high water table* [a] *on the penetration of mercury in mercuric chloride plus sodium chloride solution*

Method of application	High-water table or otherwise	Depth of penetration on succeeding days after treatment						Soil moisture
		1 day	2 days	3 days	4 days	6 days	7 days	
Set 1		*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Per cent*
1+3 [b]	High-water table			[c] 8½	9			17.9
	Checks, regular applications			[c] 9 to 10	9 to 10			17.9
Set 2								
1+3	High-water table			5 to 6	5 to 6		5 to 6	12.3
	Checks, regular applications			7+	7+		8+	17.3
	High-water table removed			5+	6 to 7		8−	12.3
Set 3								
1+3	High-water table		7 to 8	9−	8 to 9	9−		10.2
	Checks, regular applications		7 to 8	7+	6 to 7	7 to 8		10.6
	High-water table removed		8−	8+	9−	8+		10.2
Set 4								
1+1	High-water table before applications.	8 to 9	8 to 9	8−	[d] 7+			10.66
	High-water table after applications.	6+	6 to 7	5+	6 to 7			10.66
	High-water table with applications.	6−	5 to 6	6 to 7	6 to 7			10.66

To simulate a high-water table, tubes of soil were set in beakers and water added to the beakers. Set 1: 100 c. c. water was added to beakers and soil was treated 20 minutes later, after water line showed 5 inches up from bottom. Set 2: 100 c. c. water was added to beakers and soil was treated 15 minutes later. The beakers were removed from part of tubes about 24 hours after treatment. Set 3: 100 c. c. water was added to beakers; 17½ hours later 50 c. c. more water was added to beakers and soil was treated. Beakers were removed from under part of tubes next day. Set 4: One lot had 83½ c. c. water added to beakers and was tested 2 hours and 40 minutes later. Second lot, soil was treated and water added to beakers 2 hours and 30 minutes later. Third lot, soil received chemical solution and beakers received water at same time and soil received its water application 2 hours later.

[b] For explanation of symbols see p. 332 and footnote of Table VII.

[c] Each penetration figure given is the average for 3 tubes.

[d] Two tubes showed 8 to 9 inches penetration and the other 6 to 7 inches.

LATERAL PENETRATION IN SOIL OF MERCURY IN MERCURIC CHLORIDE PLUS SODIUM CHLORIDE SOLUTIONS

Sterilization of soil by application of chemical solutions can not be accomplished in soil full of stones or other impenetrable obstacles unless the chemical reaches all spores in the soil under such obstacles. A few tests of such lateral penetration were made with 6-inch flower pots, 3⅜-inch petri-dish bases for obstacles, Leonardtown silt loam containing 12 per cent of moisture, and the regular 1+1 application of $HgCl_2$+NaCl solution. Thirty-six pots were partly filled with soil, inverted petri-dish bases placed on this soil in a horizontal position and the pots filled. In 12 pots the petri-dishes were at a depth of 2 inches; in another set of 12 the petri dishes were at a depth of 3 inches; and in the third set at 5 inches. The pots were treated with 1 per cent $HgCl_2$+5 per cent NaCl at the rate of one-half gallon per square foot and followed by an equal amount of water. Four days later the soil was carefully removed from above the petri dishes, then the petri dishes were removed and the soil that had been protected by the petri dish was tested for the presence of mercury. No positive tests were obtained. Owing to the sloping sides of the pots a relatively small circle of soil was left uncovered by the petri dish even at 2 and 3 inches, while at 5 inches the petri dish almost rested on the sides of the pot.

TABLE XV.—*Lateral penetration, under an obstacle in the soil, of mercuric chloride plus sodium chloride solution as shown by hydrogen sulphide and silver nitrate tests*

	Depth to obstacle	Lateral penetration under obstacles at 1-inch and 6-inch depths on successive days		
		After 2 days	After 3 days	After 4 days
	Inches	*Inches*	*Inches*	*Inches*
H_2S test for $HgCl_2$	1	1 to 2 (4) [a]	0 to 1 (2), 1 to 2 (2).	0 to 1 (3), 1 to 2 (1).
	6	0 to 1 (1), none (3)	None (4)	None (4).
$AgNO_3$ test for NaCl	1	1 to 2 (4)	1 to 2 (4)	1 to 2 (4).
	6	1 to 2 (4)	1 to 2 (4)	1 to 2 (4).

[a] Numbers in parentheses show the number of pots, with the lateral penetration indicated.

Pots, 8-inch; petri dishes, 3½-inch; soil, Leonardtown silt loam containing 13.5 per cent moisture.

A second set of 24 pots was prepared in the same manner as the above but with the use of 8-inch instead of 6-inch pots and by placing the petri-dish bases at 1-inch and at 6-inch depths. As shown in Table XV, there was some penetration of mercury under all of the petri dishes at the 1-inch depth, while only one positive test for mercury was obtained under the petri dishes at the 6-inch depth. The diminishing number of positive tests under the center of the petri dishes at 1 inch would seem to indicate the gradual change of the mercury to compounds not precipitated by the $H_2O + H_2S$ test used. Positive tests with $AgNO_3$ throughout the soil under the petri dishes at both the 1-inch and the 6-inch depths indicated a thorough penetration by the NaCl.

Just what is responsible for the apparently poor penetration of the mercury is not known. The percentage of $HgCl_2$ was made as low as seemed safe to insure penetration in soil free from obstacles. It is well known that lateral penetration in soil is poor. It seems quite likely that most of the mercury reaching the top of the petri-dish base remains there. Even if it all worked over the edge of the petri dish, much of it would probably go down rather than laterally, under the obstacle. It would seem probable that the amount of mercury moving back under the petri dish would be so small that reactions with soil chemicals would soon change its form so that it would not react to the $H_2O + H_2S$ test used. Much the same thing may occur in tubes of dry soil treated and allowed to stand until there is no longer a sharp line between the moist treated soil and the dry untreated soil but a very gradual transition from one to the other. Soil from the transition zone is likely to give only negative results when tested for mercury.

UPWARD PENETRATION OF MERCURY

A single experiment was run to determine the approximate upward penetration of mercury as compared to downward penetration. Of 24 tubes filled with Leonardtown silt loam containing 4.26 per cent moisture, 12 tubes were treated in the usual way, 1 + 1, and the other 12 were treated by standing them in beakers and pouring the solution and the water into the beakers. The water was not added until the solution had been taken up by the soil. Three tubes from each lot were tested on the second, third, fourth, and seventh days. The

regular tubes gave positive tests at 7 to 8 inches from the top on the second day and at 8 to 9 inches on the remaining days. All of the upward penetration tubes gave positive tests at 7 to 8 inches from the bottom except two tubes which gave positive tests at 6 to 7 inches on the fourth day.

Penetration of Mercury in Soil Allowed to Stand Three Months

If soil could be sterilized without being freshly cultivated it would often save expense and facilitate treatment. When only newly cultivated soil can be treated, every rain delays the work until the soil is again in condition to permit cultivation. It is much harder to haul loads of chemical solutions over newly cultivated soil. A single experiment was set up to give an indication of the penetration to be expected in soil permitted to stand without cultivation. As it seemed quite possible there would be a difference between soil receiving most of its moisture as rain and a soil receiving its moisture through subirrigation, the tubes were divided into two sets.

Forty-eight tubes were filled with Manor loam and set in beakers; 100 c. c. water was added to the tops of half of the tubes; 18 hours later 50 c. c. water was poured into the beaker under each tube; the remaining 24 tubes had 100 c. c. water added to the beaker for each tube and another 100 c. c. water added to the beaker three days later. Three months later these tubes were treated, half of each lot being treated 1+1 and the other half 1+0. Three tubes from each lot were tested on the second, third, fifth, and sixth days. As shown in Table XVI, the penetration of 1+0 was much better than 1+1 in the tubes that had received 100 c. c. of water on top and 50 c. c. of water at the bottom, while in tubes receiving 100 c. c. twice at the bottom the 1+1 penetrated much better than the 1+0. No explanation of this variation has been suggested. Penetration in the poorer lot in each case was about equal to that obtained in freshly prepared air-dry soil.

Table XVI.—*Penetration of mercury in mercuric chloride and sodium chlorid solutions in tubes of Manor loam allowed to stand three months after having water added to them*

Water added 3 months before treatment	Chemical treatment given	Penetration on successive days after treatment			
		2 days	3 days	5 days	6 days
		Inches	*Inches*	*Inches*	*Inches*
100 c. c. water applied to top and 50 c. c. to beaker at bottom of each tube	[a] 1+1	[b] 7 to 8 (3)	7 to 8 (3)	7 to 8 (3)	7 to 8 (2); 8 to 9 (1)
	1+0	6 to 7 (1); 7 to 8 (2)	7 to 8 (1); 8 to 9 (2)	8 to 9 (1); 9 to 10 (2)	8 to 9 (1); 9 to 10 (2)
100 c. c. water applied to beaker +100 c. c. to beaker at bottom of each tube	1+1	8 to 9 (3)	7 to 8 (1); 8 to 9 (2)	8 to 9 (2); 9 to 10 (1)	8 to 9 (2); 9 to 10 (1)
	1+0	6 to 7 (2); 7 to 8 (1)	6 to 7 (3)	6 to 7 (2); 7 to 8 (1)	7 to 8 (3)

[a] For explanation of symbols see p. 332 and footnote of Table VII, p. 339. 100 c. c. of water is equal to 1½ gallons per square foot of soil surface in the tubes used.

[b] Numbers inclosed in parentheses show the number of tubes giving positive tests at the depths indicated.

Toxicity of Mercuric Chloride Plus Sodium Chloride Solutions After Passing Through Soil

The effect of passage through soil on the toxicity of the $HgCl_2$+NaCl solution was tested with only a few tubes of heavily infected soil from Freeland, Pa. Sixteen tubes were prepared in the usual manner on January 26, 1922. Thirteen tubes were treated 1+1 and 3 tubes were treated with water plus water to serve as checks. In eight days 3 of the 13 treated tubes were tested for mercury penetration. Positive tests were secured in each inch of soil in all three tubes from the surface to a depth of 9 inches. Twenty-six days after treatment the soil was removed from all tubes and used to inoculate tubers. Pots were partly filled with uninfected soil, a tuber laid on the soil, and 1 inch of soil removed from one of the tubes was scattered over the tuber and soil. The pot was then filled to the proper height with uninfected soil. At the end of three months the potato plants were examined for wart.

As shown by Table XVII, the 90 pots inoculated with soil, from the surface to a depth of 9 inches, in the treated tubes remained free from wart infection. Of 29 pots inoculated with soil from treated tubes but from below the 9-inch depth to which the mercury had been shown to penetrate, 6 pots, or 21 per cent, showed wart. Of the 35 pots inoculated with soil from the check tubes 10 pots, or 29 per cent, showed wart. A second series of tubes gave almost identical percentages of infection. Apparently the treatment of soil with 1 per cent $HgCl_2$+5 per cent NaCl solution at the rate of ½ gallon per square foot and followed by an equal amount of water is effective to the maximum depths of penetration in the Freeland soil.

Table XVII.—*Effect of soil penetration on toxicity to the potato-wart organism of mercuric chloride plus sodium chloride solution as shown by infections of potato plants grown in pots inoculated with soil from different depths in treated tubes*

Depth from which inoculation soil [a] was taken	Inoculated with water-treated soil		Inoculated with 1+1 [b] treated soil		Depth from which inoculation soil [a] was taken	Inoculated with water-treated soil		Inoculated with 1+1 [b] treated soil	
	Plants examined	Plants showing wart	Plants examined	Plants showing wart		Plants examined	Plants showing wart	Plants examined	Plants showing wart
Inches	*Number*	*Number*	*Number*	*Number*	*Inches*	*Number*	*Number*	*Number*	*Number*
0 to 1	3	1	10	0	6 to 7	3	1	10	0
1 to 2	3	1	10	0	7 to 8	3	0	10	0
2 to 3	3	1	10	0	8 to 9	3	2	10	0
3 to 4	3	0	10	0	9 to 10	3	1	10	1
4 to 5	3	1	10	0	10 to 11	3	1	10	4
5 to 6	3	0	10	0	11 to 12	2	1	9	1

[a] Wart-infected soil from Freeland, Pa., containing 12.8 per cent moisture.
[b] For explanation of symbol, see p. 332 and footnote of Table VII, page 339.

Relation of Soil Penetration of Water Solutions to the Amount Applied

With the glass soil tubes used the penetration of water could be readily determined at any time in most soils, particularly if dry, by

the difference in color between the soil dampened by the water applied and that not yet so dampened. The penetration of mercury and of formaldehyde in water solutions was found to agree largely with this visible penetration of the water carrying them. Hence the approximate penetration of given amounts of these solutions could be determined by noting their visible penetration in soil tubes or by determining the visible penetration of like amounts of water. Wart spores have not been shown to be present below the 8-inch level in soil, and this depth is usually reached by applications of 1 gallon per square foot, the amount adopted as a standard application in our experiments. It seems quite possible that deeper penetration would sometimes be desirable and in very dry soil the amount of solution applied might need to be increased to insure a uniform penetration of 8 inches. If the depth of penetration should be found to be in direct proportion to the amount of the application, the required amount of increase in application any time the penetration was found to be inadequate could be computed immediately instead of awaiting the data from experimental tests. Since the penetration of mercury and formaldehyde solutions seems to approximate the penetration of water, water alone was used in these tests.

In order to determine whether penetration was directly proportional to the amount of liquid added or whether it became greater or less (in proportion to the quantity applied) as the quantity applied was increased, 36 tubes of Leonardtown silt loam containing 5.73 per cent moisture were used. Applications of water at the rate of 1, 2, 3, 4, 5, and 6 quarts per square foot were made, using 6 tubes for each lot. At the end of one day the penetration in each tube was determined without disturbing the tubes by measuring the distance from the surface to the very distinct water line. In tubes treated with 6 quarts per square foot the water was found to have penetrated to the bottom of the tubes. An additional quart per square foot was added to each of the other tubes immediately after the measuring was completed. One day later the penetration in these tubes was again measured. The average increase in penetration, as shown by the last column in Table XVIII, is relatively uniform. The range of increases, however, in the next to the last column shows a wide variation, from 1¼ to 3¾ inches per tube. It was thought possible that the tubes showing the greatest penetration this second day might be those that showed penetration below the average on the first day, or that the conditions that caused more than average penetration on the first day would cause an increased penetration on the second day in the same tube. Inspection of the figures failed to bear out either hypothesis. A curve plotted from any one of the columns of averages would be as regular as could be expected with such a small number of tests. The maxima in the first two range columns also show almost regular curves. In practical work the minimum penetration is the important consideration. The data of this table indicate that the minimum varies greatly and the average penetration is sometimes nearly an inch greater than the minimum. Due allowance for this would have to be made in planning treatments to secure penetration to a definite depth.

The penetrations secured in this experiment are much higher than were secured in regular treatments of Leonardtown silt loam of low moisture content (Table X).

TABLE XVIII.—*Penetration of various amounts of water in Leonardtown silt loam containing 5.73 per cent moisture*

Amount added per square foot	Penetration					
	After 1 day		One quart per square foot added after measuring and another measurement made the following day		Increased second day over first	
	Range *	Average	Range	Average	Range	Average
Quarts	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
*1	2¾ to 3½	3⅛	5 to 6¼	5½−	1¾ to 2¾	2⅛
2	4¾ to 5½	5¼	7 to 8¼	7¾−	2¼ to 2¾	2½
3	7½ to 8	7½+	9¼ to 10½	10+	2¼ to 3	2½
4	9½ to 10½	10−	11¾ to 12½	12⅛	1¾ to 3	2¼
5	9½ to 12	11	12½ to 14¼	13½−	1½ to 3¼	2¾
6	13¼ to 14	13½	Penetration through column without addition.			

* Each of the six lots consisted of six tubes.

SOIL PENETRATION OF OTHER CHEMICALS TESTED

Soil Penetration of Water Solutions of Copper Sulphate

Copper sulphate is such an old and much-used fungicide that a few penetration tests were run with it notwithstanding the well-known fact that copper sulphate solutions soon lose their copper sulphate when applied to soil. A 1 per cent solution of $CuSO_4$ failed to give a positive test after percolation[13] through 14 inches of potting soil. A 2 per cent solution failed to give a positive test after percolation through 14½ inches of Manor loam. Six tubes were used in each of the above sets. Other tests with varying strengths of $CuSO_4$ seemed to indicate that attempts to get penetration with $CuSO_4$ alone in a solution were certain to fail. Since the addition of NaCl to solutions of $HgCl_2$ insures mercury penetration, it was thought that similar results might be secured by adding a salt to $CuSO_4$ solutions.

The first test of $CuSO_4$ with a salt was made with six tubes of air-dry Leonardtown silt loam, three treated 1+1 with 2 per cent $CuSO_4$ +10 per cent NaCl and three treated 1+1 with 2 per cent $CuSO_4$+ 5 per cent Na_2SO_4. At the end of four days the tubes treated with $CuSO_4$+NaCl gave positive tests for copper at 6 to 7 (2 tubes) and 7 to 8 (1 tube) inches and the tubes treated with $CuSO_4$ gave positive tests at 4 to 5 (1 tube) and 5 to 6 (2 tubes) inches. The results seemed very encouraging, especially those obtained with NaCl, so that the experiments were continued.

The results obtained with Na_2SO_4 were so poor that few tests were made with it.

The penetration of $CuSO_4$+NaCl was tested in 74 additional tubes with Leondardtown silt loam containing from 10 to 21 per cent moisture. Applications were made at the rate of 1 gallon per square foot in each case. The penetration obtained in these tubes ranged from 3 to 4 inches up to 9 to 10 inches, distributed as follows: 3 to 4 inches (4 tubes), 4 to 5 (12 tubes), 5 to 6 (19 tubes), 6 to 7 (27 tubes), 7 to 8 (11 tubes), 9 to 10 (1 tube). The penetration secured did not warrant the hope that the copper sulphate and salt solution could be expected to give adequate soil penetration to permit its use as a fungicide for potato wart.

[13] In percolation tests tubes were kept continuously supplied with solution until liquid began to drop at the bottom of the tubes.

Penetration of Qua-sul

Qua-sul is a proprietary alkaline sulphur compound said to have given good results in various types of treatments. Enough 1 per cent solution of Qua-sul was supplied to six tubes of potting soil to secure about 50 c. c. of percolate from each tube. All tests for sulphur in this percolate were negative. The soil columns were 13 to 14 inches high and about 3 gallons of solution per square foot was used to secure the percolate desired.

Penetration of Alcohol

Ordinary grain alcohol was diluted to 47 per cent alcohol and supplied for six tubes of potting soil until about 50 c. c. of percolate was obtained from each tube. The soil columns were 13 to 14 inches high and about 3 gallons of solution per square foot was used to secure the percolate desired. A strong positive test was secured in the percolate from each tube.

Penetration of Kerosene

The penetration of kerosene was tested, using Leonardtown silt loam with varying amounts of moisture from air-dry to 21 per cent moisture. The data from these experiments have been assembled in Table XIX. It will be seen that in air-dry soil the penetration of kerosene applied at the rate of 1, 2, or 4 quarts per square foot is little better than the penetration of an equal amount of water. In soil containing 10 per cent of moisture the penetration nearly doubled. Further marked increases in penetration are shown at 13, 15, 16.4 per cent of moisture. In soil containing 19 to 21 per cent of moisture the penetration of kerosene applied at the rate of 1 pint per square foot is equal to the penetration of 1 gallon of kerosene or of water in soil containing 1.83 per cent of moisture, and is equal to the penetration of 1 gallon of a water solution in soil containing 19 per cent of moisture. The penetration of 1 quart or more of kerosene in soil containing 19 per cent or more of moisture was not determined as it exceeded the height of the soil columns.

TABLE XIX.—*Penetration, in inches, of kerosene in Leonardtown silt loam on successive days and with different soil moistures*

Soil moisture	Application per square foot	1 day after treatment	2 days after treatment	3 days after treatment	4 days after treatment	5 days after treatment	6 days after treatment	7 days after treatment	8 days after treatment	11 days after treatment
Per cent	*Quantity*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
1.8	1 quart	[a] 2¾	2⅞	3¼	3					
1.8	2 quarts	4½	5½	5⅝	5¼					
1.8	4 quarts	8½	9	9	8¾		To bottom in some tubes.			
10	1 pint		2½	2½						
10	1 quart		4.9	4.9						
12	1 pint						6—	5—	7 to 8	4+
12	1 quart						8 to 9	9—	10 to 11	8+
13	1 quart		6¼	6½	6½	6⅞				
15	1 pint			6+	7+	7+		8+		
16.4	1 quart			10—	10+	11—		11—		
19	1 pint	7½	8¼	8¾	9¾					
19	1 quart	11 to 12	12+	11 to 12+	11 to 12+		All penetrated to bottom.			
20.8	1 quart		10 to 11	10 to 11	10 to 11	10 to 11				
[b]	1 quart	10+	13 to 14	13 to 14	12 to 13					
[b]	2 quarts	13 to 14	13 to 14	13 to 14	13 to 14					
[b]	4 quarts	13 to 14	13 to 14	13 to 14	13 to 14					

[a] Each figure is the average of 3 tubes.
[b] These tubes were saturated with water and then drained before kerosene was added.

Attention is called to the remarkable increase in the penetration of kerosene as the percentage of moisture increased. Kerosene, when not present in excess, has a tendency to spread over the surface of water in a rather uniform film. In very dry soil the film of moisture on the soil particles will be so thin that the exposed surface of the film will extend almost to the points of contact with adjoining particles. A slight increase in the thickness of the water film would cause a very marked decrease of exposed surface in that part of the film in the acute angles near the points of contact. It is conceivable that the increase of 1,000 per cent (1.83 to 19.0 per cent) in the amount of moisture in the soil reduced the amount of exposed water surface so greatly that the 1 pint of kerosene covered this exposed surface with a film of approximately the same thickness as that obtained with 1 gallon in the dry soil. At any rate, it seems likely that changes in the amount of water surface and of surface tension are involved in the changes in kerosene penetration.

FIELD NOTES ON PENETRATION

A considerable number of field plots were treated with various chemicals, some of them liquids or in solution. No systematic effort was made to obtain data on the penetration of these chemicals and solutions. Where penetration was extremely slow or practically failed the fact was usually recorded. These scattered notes are here summarized (Table XX).

TABLE XX.—*Readiness of penetration of chemicals in field plots*

Chemical	Penetration	Chemical	Penetration
Alcohol	Good.	Javelle water	Poor.
Bichloride of mercury solution	Good.	Kerosene	Excellent.
Bordeaux	Very poor.	Lime-sulphur	Fair.
Woburn Bordeaux	Poor.	Crude oil	Excellent.
Carbolic acid (crude)	Poor.	Potassium permanganate	Poor.
Carbolic acid solution of crystals	Poor.	Pyroligneous acid	Fair.
Chloride of lime	Poor.	Qua-sul	Good.
Cleaning solution	Very poor.	Sodium chromate	Good.
Creosote	Good.	Weed killer	Very poor.
Formaldehyde	Good.		

APPLICATION OF PENETRATION DATA TO FIELD WORK

The study of the penetration of chemicals in the soil was undertaken to enable us to intelligently plan experiments looking to the extermination of the potato wart organism in those regions of Pennsylvania, Maryland, and West Virginia in which it is known to occur. There is a considerable variation in the soils of these regions and in the amount of moisture present in them at different times and seasons. It seemed likely that these and other variable factors would largely influence the possible effectiveness of treatments. The results herein recorded give a valuable insight into the influence of some of these variables and indicate that with data from more extended experiments it would be possible to plan soil treatments with as much assurance as spraying or other remedial or preventive measures are now planned.

The influence of soil type, as shown in Tables VI, VII, VIII, IX, and X, is apparently not very marked in soils of similar nature, although the influence of the percentage of moisture present varied

in the Manor loam and Leonardtown silt loam. The results with potting soil were very erratic, possibly because of the presence of excess humus or of chemical fertilizers. The data on the influence of the size of soil particles indicate that there would have to be a very decided difference in the proportions of very coarse or o very fine particles to markedly influence penetration. Hence if penetration in preliminary tests varies greatly from that expected it seems more likely that the chemical composition of the soil is responsible than that it is due to a variation in physical make-up too slight to be noticeable to the eye.

Soil moisture is constantly changing in the regions where wart is prevalent, because of alternating spells of rain and of dry weather. As the percentage of moisture present has a marked influence on penetration, it would be necessary to determine the soil moisture at frequent intervals in order to give the most economical treatment and to insure proper penetration. Where possible, it would be advantageous to treat the soil while wet or just previous to rainy weather, as less water and materials would be required for adequate penetration. As shown in Tables VII and IX and in the discussion on the effect of applying all of the chemicals in part of the water and following with the remainder of the water as such, the penetration is improved by following this procedure. This indicates that a heavy rainfall within a reasonable time after the application of the strong chemical solution might eliminate the necessity of applying the water. Autumn applications, particularly, might eliminate the expense of much of the water. The application of chemical solutions requires special apparatus and the exercise of considerable care to secure even distribution. Relatively simple apparatus can be used in applying water. Laborers would demand more pay for handling any of the dangerous chemicals than for handling water. One man with a hose could probably apply a much larger quantity of water than one or more men operating an expensive chemical outfit even if there were no delays to refill the chemical outfit.

Much stronger tests are obtained in the soil near the line of maximum penetration when part of the water is applied after the chemicals have been applied. Tests made throughout the soil penetrated indicate a much more even distribution of the chemicals by this method. Hence it might be possible to use a more dilute initial solution at a great saving in chemicals.

The tests of penetration in tubes having a high-water table were made because some infected soil is bottom land with a water table near the surface. It was thought that adequate penetration in such land would be impossible as long as the water table remained high. The results obtained in the laboratory indicate that where this water table was stationary or receding, satisfactory penetration could be secured. The solution might not be effective much below the actual water line, however, as it would soon become greatly diluted even if the depth of penetration were adequate.

Some of the wart-infected soils contain rocks, stones, or débris. If a solution applied to these soils would not penetrate to spores underneath such obstacles, extermination by chemical treatments would become correspondingly complicated. The few experiments on lateral and upward penetration were performed to get information on the amount of penetration under obstacles. The results were

unsatisfactory. In newly prepared dry soil a considerable amount of penetration under an obstacle can be expected. The amount of chemical carried by the solution drawn under the obstacle may easily be insufficient to treat adequately so much soil. In field soil the upward flow of water is retarded by an obstacle with a consequent accumulation of moisture under it. It is doubtful if there would be an adequate flow of solution into this already moist soil. Thorough cultivation would tend to separate the obstacles from the moist soil underneath. Screening and treating soil would be expensive and should be avoided if possible. Further study of the effect of obstacles in soil to be treated should be made. It seems likely that treatments could be made effective without much trouble except where obstacles were larger than 2 inches in diameter or were very numerous. Enough soil contains large obstacles in quantity to make this a problem worthy of further study. Kerosene has such ability to penetrate that it might be useful for ground of this nature.

In the soil-treatment experiments in the field, as in the penetration experiments in the laboratory, the soil was newly cultivated or mixed so that the capillary tubes were broken up and the moisture content made uniform for all parts of the soil likely to be reached by the solution. In a few cases in the field formaldehyde seemed to make especially good penetration in soil that had been undisturbed for some time. The single set of 48 tubes used to test penetration in soil allowed to stand gave peculiar but encouraging results. Further experiments would be necessary to determine the effect of standing under different conditions. If soil could be satisfactorily treated without cultivation an important item of expense could be eliminated. If cultivation were unnecessary, soil could be treated more easily, especially in the spring before dry enough to work or when wet during the summer, as it would be difficult to haul an outfit over soft, wet ground.

Experiments to determine the effect of penetration on the toxicity of the solution to the potato wart organism were very encouraging. For the mercuric chloride plus sodium chloride solution the results indicated that any strength giving a positive test at the required depth, as shown by the hydrogen sulphide test, would be effective. No tests have been run with other fungicides. Of course, other fungi might require a greater strength of solution.

SUMMARY

Study of the penetration of chemicals in the soil is handicapped by lack of tests suitable for determining the presence of chemicals in soil or soil solutions.

Schryver's test was found satisfactory for formaldehyde, hydrogen sulphide for mercuric chloride and copper sulphate, and the characteristic odor for kerosene in soil solutions.

The penetration of formaldehyde is apparently equal to that of the water carrying it in solution even in dilutions of 0.05 per cent of commercial solution.

The penetration of mercuric chloride alone in solution is poor, but it has been found that the addition of sodium chloride at the rate of five parts of salt, by weight, to one part of mercuric chloride induces penetration usually equal to that of the water carrying the

chemicals. The solution must contain 0.5 per cent of $HgCl_2$ in order to insure such penetration in Manor loam and in Leonardtown silt loam.

The penetration of copper sulphate alone in solution is extremely poor. Penetration is somewhat improved by adding sodium sulphate to the solution, and greatly improved by adding five parts of sodium chloride to one of copper sulphate. The penetration secured was markedly less than that of the water of the solution even with solutions containing 2 per cent of copper sulphate.

The penetration of kerosene is variable, increasing remarkably with increases in the percentage of moisture in the soil. Kerosene was used undiluted.

The mercuric-chloride plus sodium-chloride solution was used in most experiments; the penetrations secured with applications of 1 gallon per square foot were as follows:

In potting soil, penetration, as shown by chemical tests, was erratic, probably owing largely to differences in the chemical makeup of different lots of soil.

In Manor loam, penetration gradually increased from about 7 to 8 inches in soil containing less than 5 per cent of moisture, to 8 to 9 inches in soil with 17 to 19 per cent of moisture, and then decreased slightly in soil with 20 to 23 per cent of moisture.

In Leonardtown silt loam, penetration gradually increased from 6 to 8 inches in soil containing less than 5 per cent of moisture, to 8 to 9 inches in soil containing 15 to 20 per cent of moisture, and to 11 to 12 inches in soil containing 43 per cent of moisture.

The effect of soil moisture on penetration is not the same for all chemicals. In Leonardtown silt loam, 1 gallon of $HgCl_2+NaCl$ solution and 1 gallon of kerosene penetrated to about the same depth in soil containing 1.8 per cent of moisture. In soil containing 19 per cent of moisture, 1 pint of kerosene penetrated as deeply as 1 gallon of $HgCl_2+NaCl$ solution.

With solutions of $HgCl_2+NaCl$, the penetration obtained by applying 1 gallon of solution is less satisfactory than that obtained by applying the same amount of chemicals in ½ gallon of solution followed by ½ gallon of water. Other proportions of solution to water gave similar results. The actual depth of penetration was about the same, but the distribution of the chemical was more uniform and the tests at points near the line of maximum penetration were markedly stronger where water followed the solution.

The data obtained will be of assistance in planning field treatments and make it possible to determine in the laboratory the strength and amount of solution required for the effective treatment of a soil, taking advantage of favorable soil moisture or of rainfall and of cheaper methods.

PART IV. POTATO GROWTH FOLLOWING CERTAIN CHEMICAL SOIL TREATMENTS

As stated in Part I, many of the chemical treatments tested as possible means of exterminating potato wart resulted in more or less severe retardation of growth of potatoes. A summary of the growth of potatoes in the treated plots during three seasons subsequent to the treatments is given below. The data here recorded are for relatively strong chemicals used in large quantities. Weaker appli-

cations made in the earlier chemical treatments gave very erratic results, the minute quantities of chemical breaking down, leaching, or forming new combinations in the soil. This is due in part, as are the fluctuations in the 100 treatments made at Sandy Run in 1921 and here summarized, to the nature of the available gardens. In no case has the soil been uniform throughout a garden, but has varied in composition by reason of mixtures due to stripping and coal-mining operations, addition of soil from the woods, the dumping of ashes and garbage, and unequal applications of manure. By distributing the treatments among these variable areas,[14] however, it is believed that the results more truly represent the comparative effects of the various chemicals used, even though they are less uniform than those obtainable by selecting contiguous beds for several applications of like chemical. Prior to the use of chemicals, and again before planting each of the two following seasons, the ground was thoroughly spaded to the depth of 8 inches.

Soil treatments were applied dry or in solution as indicated in Table XXI. The chemicals applied dry were powdered to permit even distribution. Watering cans with rose tops were used for applying liquids. Two methods of application were used; either addition to the surface of the area treated, or mixing with the soil to a depth of 8 inches by spading.

Planting followed treatment after 10 days or less the first year, while no further treatment other than spading and cultivation was given the second and third years.

The tolerance of the potato plant for the chemicals used is expressed by the symbols indicated in the footnote of Table XXI. The stand, the growth of tops, and the yield of tubers are all taken into account in determining the rating. The data presented are for particular soils and conditions and no attempt is made to draw general conclusions from them. It is of interest to note that of these chemicals Bordeaux (pl. 1, D) in whatever concentration or amount tried, potassium permanganate in its higher dilution, and a proprietary compound called Qua-sul apparently were not injurious to growth in any year.

Carbolic acid and the weaker treatment with chloride of lime (pl. 1, A) showed poor growth or none in the first year and practically normal growth in the second and third. The heavier applications of chloride of lime exhibited residual toxicity the second year. Treatments with sulphur produced the reverse condition. During the first season the plots which had received 0.12 pound of sulphur per square foot made a normal growth of tops, while the 0.25 and 0.3 pound plots made poor and very poor growth, respectively. The tops died later in the season because of the increased soil acidity. A considerable number of tubers had formed, but owing to the death of the tops these remained very small. In the second and third seasons, except for a few burned sprouts in the weakest treatment, all plants were killed (pl. 2, C). In the process of oxidation the sulphur had increased the soil acidity beyond the limit of tolerance of the potato plants.

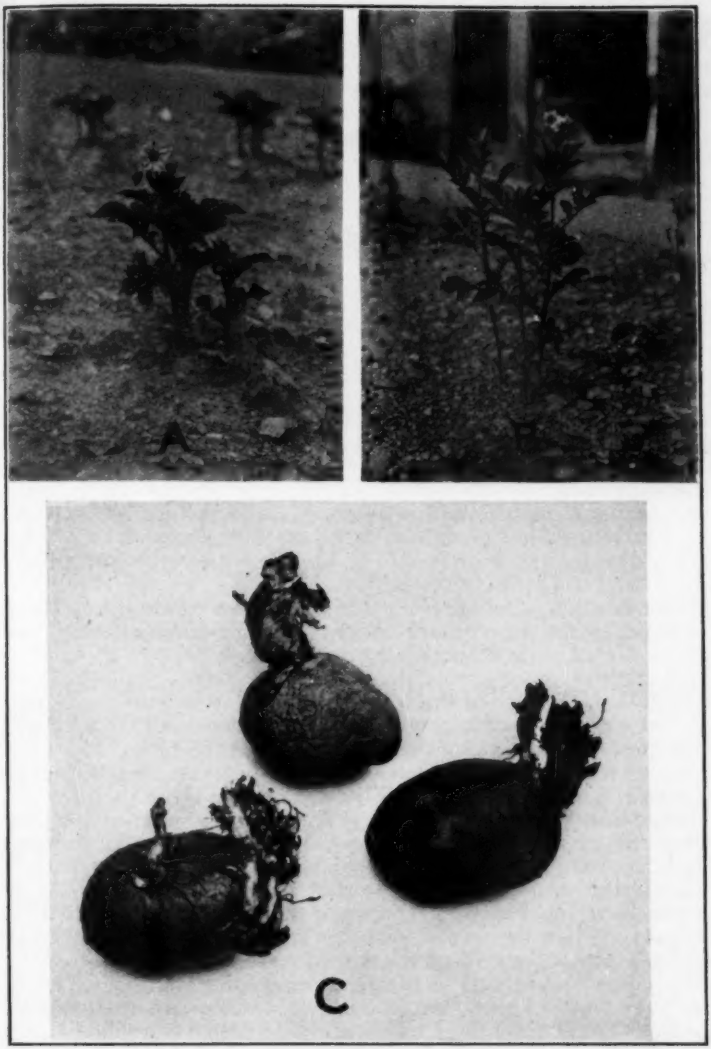
Copper sulphate (pl. 1, D), lime-sulphur (pl. 2, A), sodium carbonate, sodium chromate, sodium fluoride, a commercial weed killer, and the stronger cleaning solution treatments showed no growth in

[14] Figure 3 shows the distribution of the plots.

TABLE XXI.—*Summary of 100 chemical applications made near Freeland, Pa., 1921*

Chemicals used	Treatment applied	Amount per square foot	How applied [a]	Number of plots treated	Growth [b]		
					1921	1922	1923
Borax	Dry	0.5 to 1 pound	S and M	5	O	X to XX	XX+
Bordeaux	1 pound to 1 pound to 3 or 6 gallons	1 to 2 quarts	S and M	4	XXX	XXX	XXX
	Woburn	1 to 2 gallons	M	4	XXX	XXX	XXX
Carbolic acid	5 per cent	0.5 to 1 gallon	S	4	O	XXX	XXX
Chloride lime	Dry	0.5 pound	S and M	2	O	XXX	XXX
	Dry	1.6 pounds	M	1	O	X	XXX
Cleaning solution		2 gallons	S	1	O	O	O
		1 gallon	S	1	O	XX	XX
		1 gallon	M	2	O	(X)	O to X
Copper sulphate	Dry	0.5 to 1 pound	M	4	O	(X)	O to X
Formaldehyde	15 per cent commercial	2 gallons	S	1	(X)	XXX	XXX
	10 per cent commercial	2 gallons	S	2	X	XXX	XXX
	5 per cent commercial	2 gallons	S	2	XX	XXX	XXX
Javelle water		1 gallon	M	4	(X)	XX to XXX	XXX
Kerosene		0.8 to 2 gallons	S	4	O	X to XX	XXX
Kerosene and crude oil	1 gallon+1 gallon	2 gallons	S	1	O	XX	XXX
Lime sulphur	1 in 3 (gallons)	0.5 gallon	S and M	2	O	(X)	X–
	1 in 6 (gallons)	0.5 gallon	S and M	2	O	X	X
	1 in 12 (gallons)	1 gallon	S	1	O	X	X
Mercuric chloride	2 pounds to 25 gallons	1 gallon	S	2	O	X	X to XX–
	2 pounds to 25 gallons	0.5 gallon	S	3	O	X to XX	X to XXX
	Dry	20 grams	M	3	XX	XX	X to XXX
Mercuric chloride and salt	1 pound to 6.25 pounds to 12.5 gallons	0.5 to 1 gallon	S	4	O	X	XXX
Mercuric chloride and salt	1 pound to 25 pounds to 25 gallons	1 gallon	S	2	O	X	XXX
Oil, crude		0.5 gallon	S	2	O	X to XX	X to XXX
Potassium carbonate	Dry	0.4 pound	M	1	O	XX	XXX
Potassium permanganate	1 to 1,000	1 to 2 gallons	S	4	XXX	XXX	XXX
Pyroligneous acid		0.6 gallon	S	2	O	XX to XXX	XXX
		0.3 gallon	S	2	X	X to XXX	XXX
Qua-sul	1 in 50 or 100	0.5 to 1 gallon	S	2	XXX	XXX	XXX
Sodium carbonate	Dry	3 pounds	M	2	O	(X)	X to XX
		2 pounds	M	2	O	(X)	X
Sodium chloride	Dry	0.5 to 1 pound	M	4	O	O to XXX	XXX–
Sodium chromate	1 pound in 2 gallons	2 gallons	S	1	O	(X)	XX
Sodium fluoride	Dry	0.4 to 1 pound	S and M	4	O	O	O
Sulphur, flowers of	Dry	0.25 pound	S and M	2	X	O	O
		0.12 pound	S and M	2	XXX	O to (X)	X
Sulphur, inoculated	Dry	0.3 pound	M	1	(X)	O	X
Weed killer	10 or 20 per cent	1 gallon	M	2	O	O to (X)	O to X
Control plots		Untreated		5	XXX	XXX	XXX

[a] M = Mixed with the soil by spading. S = Surface application. [b] O = No growth; (X) = growth barely possible; X = poor growth XX = fair growth; XXX = normal growth.



TREATMENTS GIVEN IN 1921—PHOTOGRAPHS MADE IN 1922

A. Stunted growth of potatoes during 1922 in a plot treated before planting time in 1921 with lime-sulphur 1 to 5, one-half gallon per square foot. (Photographed August 10, 1922)

B. Spindling growth of potatoes during 1922 in a plot treated before planting time in 1921 with 1 per cent $HgCl_2$, 1 gallon per square foot. (Photographed August 10, 1922)

C. Growth of potatoes during 1922 in a plot treated before planting time in 1921 with 2 ounces of sulphur per square foot. (Photographed September, 1922.) The potatoes planted in the spring of 1922 failed to grow and the plot was replanted during the summer. In some cases the distorted stems did not reach the surface of the ground and few stems exceeded 1 inch in height

the first and practically none in the second and third seasons, except that the sodium chromate plot showed fair growth the third season. None of the plots treated with sodium chloride or borax produced growth during the first year. In the second season the five borax plots exhibited from poor to fair growth and in the third season exhibited fair to normal growth. Results with sodium chloride were more variable, the four plots ranging from no growth to good growth, and with no apparent cause other than that differences in soil types or drainage may have been the determining factor. Plots treated with these chemicals showed subnormal growth the third year. Pyroligneous acid, though showing much injury the first season and little the second, was highly variable in its effect on growth. During the third year normal growth occurred.

Mercuric chloride alone, and in combination with salt, except in the dry applications, killed the potatoes the first season and showed considerable toxicity the following year, both in the amount of growth which occurred and in the spindling character of plants (pl. 1, B, pl. 2, B). Growth during the third season was poor in the two plots treated with a 1 per cent solution of mercuric chloride at the rate of 1 gallon per square foot and in one of the plots treated with one-half gallon of the same solution, but was practically normal in the remaining plots.

Kerosene, crude oil, and a mixture of the two inhibited growth the first season, but permitted fair growth the second. The third season growth was poor in one of the plots treated with crude oil and normal in the other plots.

Formaldehyde showed injury during the first season (pl. 1, B), proportional to the strength of the application, and uniformly good growth for all plots the following two seasons.

All applications of the sulphuric acid and sodium chromate cleaning solution, familiar in the laboratory, used at full strength, killed the first plantings, but were sufficiently neutralized in the soil to allow some growth the following two years in two of the plots.

Javelle water practically prevented growth the first season, but fair to normal plants were produced the second and third.

When heavy chemical treatments are applied to sterilize soil, their toxicity, their alteration of the mechanical condition, and the changes which they may produce in their reaction may be injurious or not in variable degree. While it is true that as regards the growth of the potato plant the treated soil in many cases tends to return to the normal condition the second year, the reverse is sometimes the case, greater injury being evident the second season.

Therefore in applying a chemical for partial sterilization of the soil, the substance used, the rate of application, and the particular soil and climatic conditions which prevail where it is applied deserve very careful consideration from the standpoint of possible injury to plant growth as well as of its efficacy as a soil disinfectant.

(A summary is given at the end of each of Parts I, II, and III, pp. 320–321, 328–9, and 357–8).

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THE NUTRITIVE VALUE OF WHEAT: I.—EFFECT OF VARIATION OF SODIUM IN A WHEAT RATION[1]

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INTRODUCTION

The classical experiments conducted by the Wisconsin Experiment Station (*11*) with heifer calves restricted to a ration obtained entirely from the wheat plant and salt which resulted in rough-coated animals of small girth and rough in appearance as contrasted with heifers receiving a ration derived solely from the corn plant which resulted in animals in fine condition, was considered by the writers as worthy of further investigation. In a region such as eastern Washington this is especially true, for the growing of wheat predominates there and the wheat plant in many cases comprises at least the bulk of the ration for stock.

From conversations with some of the pioneers of the State of Washington who grew wheat exclusively in the early days, the writers have learned that cattle and their offspring showed no ill effects which were regarded as due to rations that were restricted to the wheat plant. This also suggested a further study with wheat. The possibility that wheat grown in the Middle West might be different in composition from wheat grown in the Pacific Coast States seemed another good reason for further investigation. It was also considered that the wheat plant might have a different effect on livestock when consumed in pasturing than when consumed as mature grain and straw.

In 1919 work was started by the senior writer to try to determine if possible why the Wisconsin Experiment Station and the practical feeders of the Northwest obtained different results. In 1921 the junior writer took charge of the work and started a project on the nutritive value of wheat and wheat products. This was designed to study the nutritive deficiencies of wheat, limits in the use of deficient food elements, variation in the nutritive value of wheat and the factors on which variation depends, relative nutritive value of different varieties of wheat, and the nutritive value of the flour from the wheats studied. This paper is a progress report on a part of the work done.

REVIEW OF LITERATURE

According to Hart, McCollum, and collaborators of the Wisconsin Experiment Station (*11*),[3] a ration restricted to the wheat plant did not seem to allow successful growth and reproduction. In a later publication (*13*), they expressed the opinion that the inferior results with the wheat-plant ration were probably due to mineral deficiency, and inherent toxicity in the wheat kernel, with possibly a third deficiency—vitamin A.

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[3] Reference is made by number (italic) to "Literature cited," p. 374.

After some experiments on feeding swine a large amount of wheat, Hart, Miller, and McCollum (*12*) state that toxicity follows even in the presence of all the recognized factors for growth. Apparently in a like manner, wheat has seemed to others (*3*) to cause injury to the progeny of rats.

It has long been known that cereals in general, and wheat in particular, contain inadequate amounts of the elements sodium, chlorine, and calcium, when compared with the quantity contained in milk (Tables I and II). In feeding animals there is a greater probability of feeding insufficient sodium than potassium, which is in predominant quantity among the minerals in the cereals (*27*).

TABLE I.—*Mineral constituents in cereals and roughage*

Kind of cereal	Per cent, dry basis				
	Ash	K	Na	Ca	Cl
Wheat (4)	1.866	0.59	0.035	0.056	0.095
Wheat (27)	----------	.515	.0106	.044	.088
Wheat (27)	----------	.431	.051	.044	.080
Corn (4)	1.410	.396	.030	.014	.073
Oats (4)	3.709	.460	.184	.112	.077
Wheat flour (4)	.192	.058	.127	.022	.081
Wheat flour (27)	----------	.146	.069	.026	.076
Wheat straw (4)	3.65	.842	.237	.217	.209
Corn stover (4)	7.007	1.847	.065	.507	.308
Alfalfa hay (4)	6.89	.832	.489	1.130	.161

TABLE II.—*Mineral constituents in milk* (*2*), (*17*)

Kind of milk	Per cent, dry basis (calculated)				
	Ash	K	Na	Ca	Cl
Cow	5.75	1.22	0.59	0.98	1.13
Goat	5.45	.76	.35	.99	.72
Human	1.68	.56	1.60	.29	.39
Sheep	4.39	4.23	.33	.91	.68
Horse	4.34	.94	.11	.97	.34

The mineral analyses of the various parts of the body show that potassium predominates in the muscles, brain, liver, saliva, and milk; and sodium in blood, serum, lymph, pancreatic fluid, bile, and gastric juices. Bone is higher in sodium than in potassium.

The amount of sodium in the ash of wheat is reported from a trace to a fraction more than 7 per cent. In the writers' analyses the sodium is found to exceed 12.5 per cent of the total ash. The amount of potassium is comparable with the results reported by other investigators (*15*). The percentage of calcium is about the same and the chlorine less in the wheat analyzed and used by the writers.

The percentage range of the minerals in wheat may be summarized as follows: Iron and aluminum, 0.27 to 0.40; potassium, 0.34 to 0.49; magnesium, 0.075 to 0.14; calcium, 0.38 to 0.63; phosphorus, 0.36 to 0.47; sulphur, 0.036 to 0.083; and sodium, a trace to 0.23.

In many feeding trials calcium has been included, and sodium chloride has been given *ad libitum;* or salt mixtures have been pre-

pared with varying proportions of different kinds of salts, among which are salts containing calcium, sodium, and chlorine (*19, 20, 23, 24*). However, the inclusion of these elements with the restricted wheat ration and vitamin A, or wheat in combination with wheat gluten and vitamin A, have not met the needs of the mother rats in caring for their young in the Wisconsin experiments.

In the following pages data will be presented showing that rats can be successfully fed rations similar to those used at Wisconsin, and that through certain adjustments in making up the mineral deficiencies these rats can properly care for their young and successive generations may be brought to maturity. The effect of the variation of the amount of sodium in such a wheat ration is also shown.

With the exception of calcium and phosphorus (*5*), comparatively little work seems to have been done to determine the optimum amounts of the various inorganic elements which are needed by animals under varying conditions. Even the work on calcium and phosphorus is far from complete. Forbes (*2, p. 145*), in 1909, made the following statement:

> Our knowledge of the amounts and kinds of mineral matter required by animals is indefinite and fragmentary. Much progress has yet to be made in this field. Such recommendations as we are able to make should be regarded only as general indications of the truth.

Forbes has since made an extensive study of phosphorus (*5*) and calcium, as reported in Ohio Agricultural Experiment Station bulletins (*6, 7, 8, 9, 10, 25, 26*) and in other papers. Sherman (*27*) also has recognized the need of a further quantitative study of the inorganic elements.

Emphasis has been placed upon the acid-base balance (*27, 3*) of inorganic elements. Later information (*15, 16*) tends to show that excess of mineral acids is not as harmful as was formerly believed. Lamb and Evvard (*16*) make this statement: "If the other elements in a natural ration are satisfactory, it is not necessary to balance the acid and basic mineral elements for growing swine."

That there is lack of definite information regarding the inorganic elements in human and animal nutrition may be shown by an examination of such texts as those by Lusk (*18*), Henry and Morrison (*14*), and Armsby (*1*).

Harnemann, quoted in Lusk, states that in man the sodium-chloride balance was maintained with 5 gms. of salt daily, which was half to one-quarter the amount usually taken. Lusk states that "one of the most important questions of the time concerns the determination of the quantity of salts in the food necessary to prevent malnutrition in children."

Henry and Morrison state in 1922 (*14*) that "at present there is little data regarding the minimum amounts of lime and phosphorus which will permit normal development of growing animals." They say that a reasonable allowance of salt for a horse is 2 oz. per day; and that milk cows should receive at least 1 oz. a day; that 0.75 oz. a day per 1,000 lbs. of live weight with 0.3 oz. in addition for every 10 lbs. of milk produced is generally sufficient. "A plan followed by many dairymen is to mix 0.5 lb. to 1 lb. of salt with each 100 lbs. of concentrates, and then in addition to provide salt so the cows can have access to it and take all they wish."

In trials at the Iowa Experiment Station during five years, Evvard found that 2-year-old steers fattened on typical Corn Belt rations, including corn silage, and allowed free access to block salt, consumed just about one-third ounce of salt per head daily. The gains of the animals were very satisfactory. "In a trial carried on by the Kansas station it was found that during the summer yearling and 2-year-old steers on pasture licked about 1 oz. of salt per head daily from salt blocks placed in the pasture" (*14*). "In an experiment in France, sheep fed 0.5 oz. of salt daily with their feed gained materially faster than those fed no salt, and also somewhat more rapidly than others fed 0.75 oz. daily. The fleeces of the salt-fed sheep were of better quality and heavier than those of the sheep fed no salt" (*14*).

Armsby emphasizes the need of further study of the inorganic elements. He feels that the amount of these elements actually necessary is less than is often supposed. The data which he gives regarding sodium, potassium, and chlorine, seem to be conflicting.

An investigator in 1907 (*21*) stated that the ability of the body to take up sodium salts is practically unlimited. He speaks of 12 to 15 gms. of sodium chloride as the normal amount used by man, and 7 to 8 gms. as the normal amount on a milk diet.

EXPERIMENTAL DATA

In the writers' preliminary experiment 9 young white rats were divided into 3 lots and fed the same basal ration, consisting of 100 gms. of ground wheat, 10 gms. of wheat gluten, and 5 gms. of prepared butterfat. The ration contained approximately 16.6 per cent of protein and should satisfy the requirements of the white rat as far as protein, fat, carbohydrate, and vitamins are concerned. The food given to lot No. 1 consisted of the basal ration plus 0.5 gm. sodium chloride. For the second lot 0.5 gm. calcium chloride was added to the basal ration, and for the third lot, a combination of 0.5 gm. sodium chloride and 0.5 gm. calcium chloride was given with the basal ration.

Two of the rats given the rations containing the sodium chloride died at 4 and 5 weeks respectively, while the remaining one developed slowly but very unsatisfactorily. Likewise, two of the rats given the calcium chloride and sodium chloride combination died at 3 and 11 weeks, and the one remaining made somewhat more rapid development than the rats given the sodium chloride. The rats fed the calcium-chloride and sodium-chloride combination seemed to withstand approximately double the quantity of chlorine present in the sodium-chloride ration. The lot receiving the calcium-chloride ration not only made better gains than was noted in case of either of the other two lots, but also without mortality.

The female in the calcium-chloride lot finally raised six young rats successfully. Toward the end of the nursing period, the mother showed signs of becoming blind, and her head was twisted to one side. She conceived again but aborted. In the third gestation period much difficulty was experienced at parturition. At this time there was no doubt as to the effect produced on the nervous system, and blindness was apparent. The mineral content of the ration for this rat was then changed to 0.1 gm. sodium chloride with 0.4 gm. calcium chloride, when she again successfully reared two litters of seven and four young, respectively.

The first young rats from this calcium-chloride mother, and two young stock rats, were divided into two lots, one of which (lot A) received the calcium-chloride ration, and the other (lot B) received the 0.1 gm. sodium chloride and 0.4 gm. calcium-chloride ration. In this plan it will be seen that the probability of the transmission of any beneficial stabilizing influence which might have originally been present through the use of other foodstuffs was greatly reduced.

In the remaining six rations of this experiment 0.5 gm. of calcium chloride and a varying amount of sodium in the form of sodium bicarbonate were added to the basal ration as follows: Per cent, Na: (A), 0.228; (B), 0.260; (C), 0.287; (D), 0.354; (E), 0.525; (F) 0.785; (G), 1.29; and (H), 2.58. The rations contained 0.21 per cent calcium and 0.27 per cent chlorine. In all other respects the rations were alike.

The wheat was finely ground. The gluten used had the qualities specified by the patent taken out by one of the writers (*22*). The butterfat was carefully prepared by filtering out the casein and washing out the salts.

Figure 1 gives the composite growth curves for the eight different rations.

The curve for ration A is the average of four closely agreeing curves and it will be noted that it is decidedly flat in comparison with the normal growth curve. There was no successful production of young on this ration and apparently only one conception.

The curve for ration B shows much better growth than for A, and in fact follows the normal-growth curve for more than four weeks. Young were successfully produced on this ration, and they in turn produced a third generation.

For ration C the curve shows better growth than for B, as it quite closely approaches the normal-growth curve for 16 weeks. One vigorous litter of young was produced and later a second litter by the same mother. Another female produced seven young which were all lost. The first litter mentioned produced a third generation.

The curve for ration D is practically identical with that for C for 10 weeks. Three litters of young were born within a week and a fourth shortly after. Three second litters were produced later.

The curve for E shows that this ration produced the best growth of any of the rations in this series. Vigorous young were produced on this ration.

The F curve shows a less rapid gain than the preceding rations, although the gains were the most economical, as noted elsewhere. One satisfactory litter of young was produced.

The G curve is quite similar to the F curve but no young were produced.

The curve for ration H is flat, although it is somewhat better than for A, at least up to six months. One litter of young was produced after the point at which the curve ends, when the mother was about a year old. One of these young died at 2 months and one at 3½ months, the latter weighing 32 gms. Two were alive at 5 months, one weighing 103 gms. and one 56 gms.

Figures 2, 3, 4, 5, and 6 show the composite curves for males and for females on rations A to E, respectively. In the remaining rations the two curves (not shown) are practically identical.

After the completion of the work just discussed, three of the rations, A, D, and H, were repeated with a different lot of wheat, and results supporting those just given were obtained.

The amount of feed in grams per gram of gain for the first 4½ months, and for the first 9 months is as follows:

	A	B	C	D	E	F	G	H
4½ months	40.7	12.2	11.7	9.9	8.7	7.2	10.0	16.1
months	38.1	21.7	21.1	20.5	----------	14.1	16.9	22.6

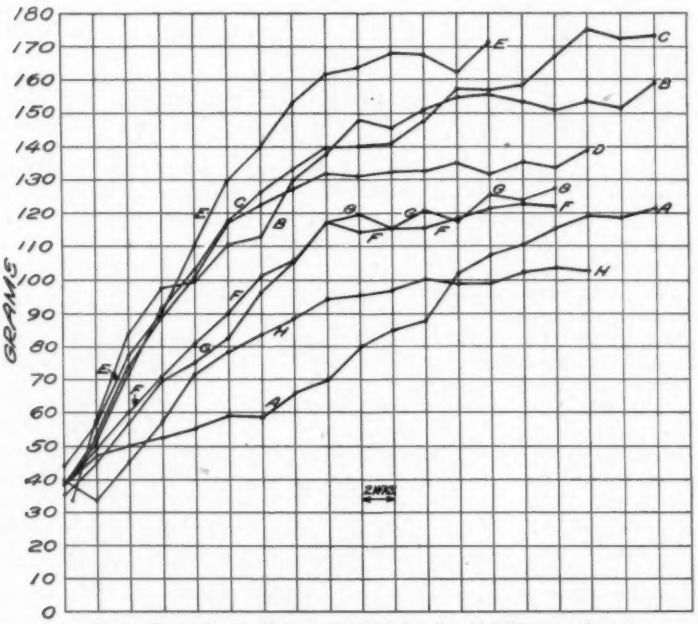

FIG. 1.—Composite growth curves for rats fed on the eight different rations

It will be noted that the addition of a small amount of sodium to this ration greatly increased the economy in the use of the feed. Increase of the amount of sodium fed seemed to effect a greater economy, up to a certain point. But, beyond this point, additional sodium seemed to reduce the economy. Ration F containing 0.785 per cent sodium seemed to produce the most economical gains in weight. However, this amount did not seem to promote the most successful reproduction.

It is also interesting to note the amount in grams of sodium required per gram of gain:

	A	B	C	D	E	F	G	H
4½ months	0.0928	0.0317	0.0336	0.0350	0.0457	0.0565	0.129	0.4154
9 months	.0869	.0564	.0606	.0726	----------	.1107	.218	.5831

The daily consumption of feed in grams per rat was the largest in lot B, with decreasing requirements for lots C, D, and the other rations, as indicated in the following tabulation:

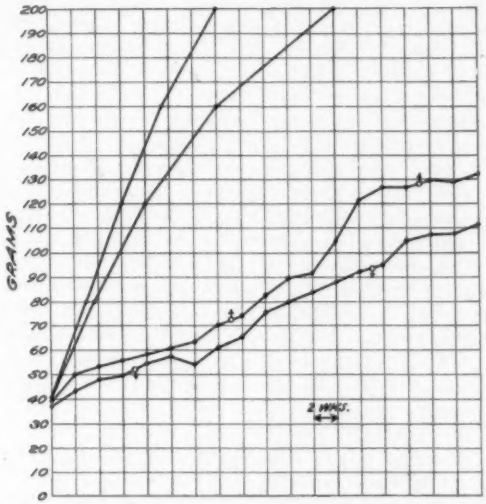

FIG. 2.—Composite growth curves for male and female rats fed on ration A. Normal growth curves also shown

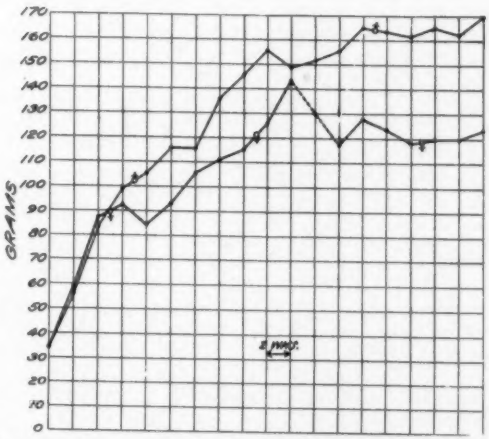

FIG. 3.—Composite growth curves for male and female rats fed on ration B

	A	B	C	D	E	F	G	H
4½ months	12.13	13.85	11.59	7.75	8.07	5.10	5.60	5.67
9 months	13.04	15.41	14.02	9.04	----------	7.04	5.82	7.51

A corresponding order of course prevails for the protein, butterfat, calcium, and chlorine intake. The amount of sodium in grams consumed by each rat each day is as follows:

	A	B	C	D	E	F	G	H
4½ months	0.0276	0.036	0.033	0.037	0.042	0.040	0.072	0.172
9 months	.0297	.040	.040	.035	----------	.055	.075	.194

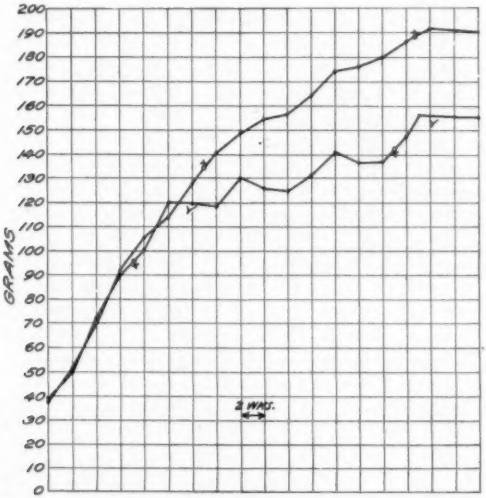

FIG. 4.—Composite growth curves for male and female rats fed on ration C

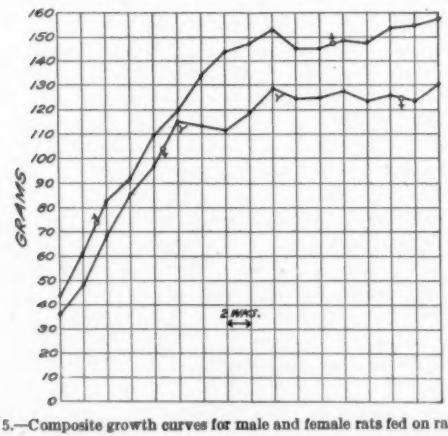

FIG. 5.—Composite growth curves for male and female rats fed on ration D

From this it would appear that approximately 40 mgms. of sodium per day is an optimum amount, in such a ration. Twelve or thirteen gms. of feed per day in ration A did not furnish that much sodium,

while 14 or 15 gms. of feed per day in B did supply that much, and much better growth and reproduction resulted. Smaller amounts of feed in rations C, D, E, and F furnished this amount of sodium, with greater economy in food consumption, there was better reproduction, and the gains were more rapid than was the case on ration A. The average daily gain in grams per rat for the two different periods is shown as follows:

	A	B	C	D	E	F	G	H
4½ months	0.30	1.13	0.99	0.78	0.82	0.70	0.56	0.42
9 months	.34	.7	.66	.48	----------	.50	.35	.33

With the larger amounts of sodium consumed per day in rations G and H, there was a lower average daily gain and a poorer economy of feed.

It is evident that the variations in growth were not due to the nature of the proteins or to lack of vitamin A or vitamin B—because the best gains resulted when the intakes of these essentials were less.

The animals on rations C and D seemed to have a tendency to produce young at an earlier age than those on the other rations. There was a marked group difference in the coats of the various lots. The hair was very fine in lot A, and coarser in lots B, C, and D. The coat of lot C was of excellent quality.

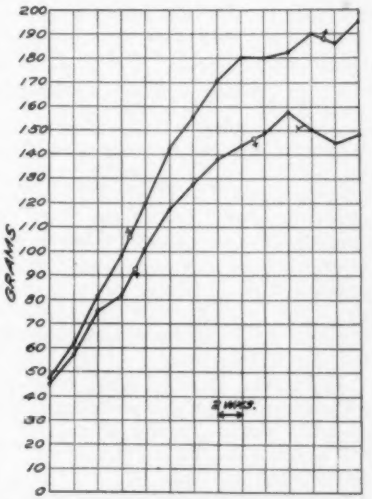

FIG. 6.—Composite growth curves for male and female rats fed on ration E

DISCUSSION

It has been found that rats not only maintain themselves but make some growth on wheat grown in the Pacific Northwest when this wheat is supplemented with gluten, butterfat, and calcium chloride. The addition of sodium seemed to make it possible for the rats to reproduce and successfully care for their young. The lot A rats were later transferred to the lot B ration, and 30 days later one of the females gave birth to four young.

Various combinations of mineral supplementation have been studied, and some of them have produced results similar to those reported by the Wisconsin Experiment Station. The altering of the mineral supplement has caused different degrees of improvement. Although the writers' work shows a higher sodium requirement than that shown by the work of some other investigators, it must be considered that the animals used in the present investigations were put on the various rations as soon as they were weaned and therefore

had less opportunity to benefit from any sodium which might be stored up by older animals. There is also no variation of other elements in these rations, and the reproduction records and the record of the total food requirement have been taken into consideration.

Analyses show marked differences in the mineral composition of wheat. These differences may have been partly responsible for the failure noted in the Wisconsin experiments. The success attained at the Washington Experiment Station following adjustments of minerals has pointed to the importance of ions rather than salts.

The success experienced in raising livestock on wheat rations on the farms of the Pacific Northwest has been believed to be due largely to the salts in the soils and water, and to the amount of mineral in the wheat. The opinion has been held that the potassium carbonate used with the Wisconsin wheat should have been replaced by sodium carbonate, or, at least, by sodium bicarbonate or some organic salt of sodium. On the basis of the results obtained here, it appears that the substitution of sodium for the potassium in the Wisconsin rations might have entirely altered the results there.

SUMMARY

A study has been made of the effect of a variation of sodium in a wheat ration on growth and reproduction. The ration containing no more sodium than the amount present in the wheat was not accompanied by normal growth or successful reproduction. The addition of sodium in the form of sodium bicarbonate was accompanied by much better growth and successful reproduction. Large amounts of sodium caused detrimental effects. From the results it appears that 0.23 per cent of sodium is too small an amount, 0.53 per cent is the most satisfactory when both growth and reproduction are considered, and that 0.785 per cent and amounts above this are detrimental.

It is also thought that a proper adjustment of the amount of sodium in the ration caused an economy in the use of feed. In the rations which proved the most successful, the animal consumed an amount of feed which furnished approximately 40 mgms. of sodium per animal.

Growth and reproduction were successful on a wheat ration which was corrected for certain nutritional deficiencies.

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AN APPROXIMATE METHOD OF CALCULATING COEFFICIENTS OF INBREEDING AND RELATIONSHIP FROM LIVESTOCK PEDIGREES[1]

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INTRODUCTION

In previous papers[2] one of the writers has described coefficients of inbreeding and relationship designed to make possible the interpretation of livestock breed histories in terms of the Mendelian theory of inbreeding and crossbreeding. The formula for the coefficient of inbreeding may be repeated here for convenience:

$$F_x = \Sigma[(\tfrac{1}{2})^{n+n^1+1}(1+F_a)]$$

In this formula, F_x is the required coefficient, and F_a is the similar coefficient for any common ancestor that makes the closest connecting link between a line of ancestry tracing back from the sire and one tracing back from the dam. The generations from sire and dam to such a common ancestor are designated n and n^1, respectively. The contribution of a particular tie between the pedigrees of sire and dam is $(\frac{1}{2})^{n+n^1+1}(1+F_a)$. There is a factor $\frac{1}{2}$ for every generation in the tie between the germ cells which unite to form the individual, reckoning the germ cells as each half a generation from sire and dam. The factor $(1+F_a)$ measures the effect of prepotency of a common ancestor that is himself inbred. The total coefficient is simply the sum of all such contributions. It is to be noted that the same animal may form the tie between many different pairs of ancestral lines of the sire and dam.

This coefficient was shown theoretically to give the coefficient of correlation between the egg and sperm which unite to produce the individual in question. As far as Mendelian factors are involved, its use implies the definition of inbreeding as the bringing together of similar germ cells. It was also shown that this coefficient measures the percentage reduction from the average degree of heterozygosis in the foundation stock. As experiments with different kinds of animals and plants have indicated that the effects of inbreeding, such as decline in vigor, fixation of type, prepotency in crosses, vary directly with the increase in homozygosis, the coefficient appears to be the proper one to use from the physiological standpoint.

It may be well to call attention to the point that the coefficient is not an absolute, but a relative, measure of a quality of an animal. It measures the probable similarity of the germ cells which united to produce him, relative to the similarity of random germ cells from the foundation stock. Random-bred descendants of the foundation stock would have a coefficient of zero, even though the foundation stock itself might be highly inbred relative to more remote ancestors.

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[2] WRIGHT, S. COEFFICIENTS OF INBREEDING AND RELATIONSHIP. Amer. Nat. 56: 330-338. 1922.
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Similarly, from the viewpoint of decrease in heterozygosis, the coefficient measures merely the change from the situation in the foundation stock toward perfect homozygosis, whether the foundation stock showed 50 per cent heterozygosis or 1 per cent or the infinitesimal amount which would doubtless be found with complete knowledge of the genetic constitution. In analyzing breed history, it is as legitimate to trace all pedigrees back to some arbitrary date as to trace them to the beginning of the herdbook. It is merely necessary to exercise due care in interpreting the results as relative to whatever foundation stock is used.

Closely related to the coefficient of inbreeding is the coefficient of relationship, which gives the degree of correlation to be expected between two individuals (X and Y) in characteristics which are entirely genetic and without dominance, conditions under which the correlation between parent and offspring or between brothers in a random-bred stock is +0.50. The interpretation, of course, depends on the choice of foundation stock.

$$R_{xy}=\frac{\Sigma\left[\left(\frac{1}{2}\right)^{n+n'}(1+F_a)\right]}{\sqrt{(1+F_x)\ (1+F_y)}}$$

In this formula F_x and F_y are the coefficients of inbreeding of the two individuals, F_a is that of the closest common ancestor connecting a pair of ancestral lines in their pedigrees, and n and n' are the number of generations from X and Y to this common ancestor along the lines in question.

APPLICATION TO BREED HISTORY

These coefficients have been applied, in a recent paper,[3] to the analysis of Thomas Bates' methods of breeding in developing his famous Duchess family of Shorthorns. The coefficient of inbreeding of each of the 64 Duchesses, and of their sires and dams, was calculated from the pedigrees carried back to the foundation stock of the breed as completely as the pedigrees are recorded in the herdbook. The degrees of relationship of all of the Duchesses, and of their sires and dams, to the bull Favourite (252), the most important foundation animal of the breed, were also found from the complete pedigrees.

The Duchesses, however, came fairly early in Shorthorn history. The longest straight female line in the pedigrees was only 13 generations. Even here it was a rather tedious process to work out the coefficients for the later animals. It is obvious that the amount of work would become practically prohibitive in dealing with the breeds as they are to-day. The complete pedigree of a modern Shorthorn would require the tabulation of several million names. Fortunately, there is a very simple approximate method, the results of which can be brought as close as desired to the complete method.

This approximate method, which it is the purpose of the present paper to describe, rests on the tabulation of random samples of the pedigrees of sire and dam. The reliability of the results can be tested by the ordinary theory of sampling.

[3] WRIGHT, S. MENDELIAN ANALYSIS OF THE PURE BREEDS OF LIVESTOCK. II. THE DUCHESS FAMILY OF SHORTHORNS AS BRED BY THOMAS BATES. Jour. Heredity 14: 405–422, illus. 1923.

It is necessary that the sample lines be chosen wholly at random. For obvious reasons, common ancestors are more likely to be males, in livestock breeding, than females. Thus straight male or straight female lines can not give a fair basis for calculating the number of ties between two pedigrees.

A system of alternating male and female lines has been tried out by the writers; and, while it gives fairly satisfactory results, the theory of sampling can not be applied in a simple way to calculate a probable error. If it could be calculated, the probable error would undoubtedly be somewhat larger than where truly random lines are used. With this or any other regular sequence of sires and dams the same lines tend to recur in different pedigrees and contribute more or less than would a random line, depending on whether important foundation animals happen to be represented in them or not.

A truly random line of ancestry can be obtained by letting the sequence of sires and dams which is to be traced back in the herdbook be that of the heads and tails, respectively, in a coin-tossing experiment. The same sequence should not, of course, be used frequently in the same study. The writers use a record of 1,000 coin tossings as material for obtaining random sequences. The sequence for each new line is begun where the last leaves off. A given sequence has little chance of repetition, since the number of generations in the lines varies.

The simplest possible sample which can show a connection between sire and dam is obtained by tracing back two ancestral lines, one on the sire's side and one on the dam's side. The scheme is illustrated in Tables I and II.

TABLE 1.—*A two-line pedigree sample of the Shorthorn bull Millionaire (79438)*

[An example of a case in which an ancestral connection is shown]

MILLIONAIRE (79438)

White Bear (75896)	Granny's Wealth
D Amethyst	S Sign of Riches (66324)
D Artless	S Lord Mayor (61193)
D Amy	D Lady Mysie 5th
S Grand Duke 36th (43306)	S Field Marshal (47870)
S Grand Duke 30th (38373)	S Roan Gauntlet (35284)
D Grand Duchess 24th	D Princess Royal
S 3d Duke of Clarence (23727)	S Champion of England (17526)
D Duchess 80th	D Virtue
D Duchess 72d	S Plantagenet (11906)
D Duchess 67th	S Duke of Lancaster (10929)
D Duchess 59th	S Duke (9032)
S 2d Duke of Oxford (9046)	D Hartforth Cherry
D Oxford 2d	S Major Bown (2243)
S Short Tail (2621)	S Charles II (879)
S Belvedere (1706)	S Percy (1321)
D Angelina 2d	S Charles (127)
S Young Wynyard (2859)	D Cora
S Wellington (680)	D Maria
S Comet (155)	D Gaudy
S Favourite (252)	S Favourite (252)
S Bolingbroke (86)	D Phoenix
D Young Strawberry	D Favourite Cow
D Favourite Cow	S Alcock's Bull (19)
D Unknown	S Unknown
S Smith's Bull (608)	

TABLE II.—*A two-line pedigree sample of the Shorthorn bull Scottish Victor (79925)*

[A case in which no ancestral connection is shown]

SCOTTISH VICTOR (79925)

Cairo (72151)	Stella 2d
S Prince of Fashion (64597)	S Matchmaker (69105)
D Primrose 3d	S Pride of the Morning (64546)
S Norseman (56233)	S Star of the Morning (58189)
D Nonpareil 20th	D Baronness 6th
S Cumberland (46144)	S Field Mashal (47870)
S Pride of the Isles (35072)	D Azalea
D Golden Days	S Caesar Augustus (25704)
S Lord Raglan (13244)	S Champion of England (17526)
D Brenda	D Virtue
S Maunby (7223)	S Plantagenet (11906)
S Clementi (3399)	D Madaline
D Cassandra	D Landlady
D Garland	D By Pilot (496)
S Matchem (2281)	S Pilot (496)
D By Farmer (251)	D Red Rose
S Farmer (251)	S By Punch (531)
S Bumper (101)	D By Broken Horn (95)
S Windsor (698)	S Broken Horn (95)
S Favourite (252)	D By Hubback (319)
D Phoenix	D By Bank's Bull
S Foljambe (263)	S Bank's Bull
D Haughton	
D By Colling's Bull	

In the case of the Shorthorn bull Millionaire (79438) (Table I), the sire (White Bear 75896) and dam (Granny's Wealth) are both recorded. Random sequences of S's and D's are then written in columns below each parent, extending sufficiently to include the foundation stock. The line of ancestry is then traced back in the herdbook, the sire being looked up where S occurs in the column and the dam for each D. In the case of White Bear (75896), the sequence happened to run DDDSSD . . . , etc. Thus White Bear's dam, Amethyst, is recorded, but not his sire. Then follow *her* dam, Artless, *her* dam, Amy, *her* sire, Grand Duke 36th (43306), and so on until the herdbook fails. A single random line of ancestry of Millionaire's dam is tabulated in the same way, the sequence in this happening to run SSDSSDS . . . Of course a second sample of the ancestry of the same animal would probably not show the same sequence of sires and dams, and it may be well to make clear that a single sample of this sort is of practically no value as an indication of the inbreeding of an individual. But the average obtained from a large number of such samples should not differ appreciably from the true value.

Two-column samples of this kind fall at once into two alternative categories; those which show an ancestral connection, as Millionaire (79438), and those which do not, as Scottish Victor (79925), Table II. In the latter case the coefficient is zero as far as the sample indicates. In the former case a contribution of $(\frac{1}{2})^{n+n^1+1}(1+F_a)$ is indicated if the common ancestor A is n generations back of the sire and n^1 back of the dam. The sire has 2^n ancestors in the nth generation and the dam 2^{n^1} in the n^1th generation. The sample pair of lines is thus only one among 2^{n+n^1} possible pairs going back as far as the common ancestor. If the single pair of lines is a fair sample of the total, its contribution must be multiplied by 2^{n+n^1} to obtain an estimate of the inbreeding of the whole pedigree. On carrying out this multiplication, n and n^1 disappear, and the coefficient takes the simple form $\frac{1}{2}(1+F_a)$. Thus, in calculating the

inbreeding indicated by a two-column pedigree, it is not necessary to count the generations to the closest common ancestor; it is merely necessary to note whether there is a tie, and what animal is responsible for it. Neglecting for the moment the effect of inbred common ancestry, the coefficient merely takes the values 50 per cent and 0 per cent under the two alternatives. As noted before, such a determination means practically nothing as far as the individual is concerned. But by determining the proportion of such ties in a sufficiently large random sample of a family or breed, a measure of the average degree of inbreeding of that family or breed can be obtained to as high a degree of accuracy as desired. If, for example, 40 two-column pedigrees show a common ancestor and 60 do not, an average inbreeding of 20 per cent ($=40\times0.50$) is indicated, again neglecting the term $(1+F_a)$.

The probable error of the percentage of ties can be calculated by the usual formula, $E_p=0.6745\sqrt{\frac{pq}{N}}$, where N is the number of cases, p is the observed chance of occurrence of a tie, and q $(=1-p)$ is the chance of nonoccurrence. In the case cited, $E_p=0.6745\sqrt{\frac{0.40\times0.60}{100}}$ $=0.032$. As 40 per cent of ties corresponds to an inbreeding coefficient of 20 per cent, the probable error of the latter must be rated down proportionately, giving 20 ± 1.6 per cent. Allowance for the factor $(1+F_a)$ will be considered later.

The method may be used to calculate the inbreeding coefficient of an individual, by finding the percentage of ties in a large number of random two-column samples from his pedigree. Increased accuracy may be obtained in this case, however, by a combination of the approximate method with the complete method. The pedigree may, for example, be tabulated completely for five generations, continuing each of the 32 lines to the foundation stock by the random method. As there are 16 random lines back of the sire and 16 back of the dam, there are 256 tabulated pairs of lines to be considered for possible ties. The total number of possible pairs of lines tracing n generations back of the sire and n^1 back of the dam is thus $\frac{2^{n+n^1}}{256}$ times the sample, if n and n^1 are both greater than four in this case. The contribution of an observed tie this distance back in the pedigree is $(\frac{1}{2})^{n+n^1+1}$ $(1+F_a)$. Multiplying by the number of pairs of lines which it represents gives $\frac{1}{512}$ $(1+F_a)$ as the contribution to be assigned the observed tie as a sample of the complete pedigree. This expression, it will be noted, is free from n and $n,^1$ as in the two-column case. It is easy to see that, in general, the contribution to be assigned to a tie in the random part of a pedigree which is complete for k generations back of the parents is $(\frac{1}{2})^{2k+1}$ $(1+F_a)$.

If a common ancestor appears in the complete portion of the pedigree on one side and in the random portion on the other side, this formula requires modification. Letting n be less than k but n^1 greater, the ratio of possible to tabulated pairs of lines tracing n generations back of the sire and n^1 generations back of the dam is $\frac{2^{n+n^1}}{2^{n+k}}=\frac{2^{n^1}}{2^k}$, instead of $\frac{2^{n+n^1}}{2^{2k}}$, as in the case previously considered.

Multiplying by $(\frac{1}{2})^{n+n'+1}(1+F_a)$ gives $(\frac{1}{2})^{n+k+1}(1+F_a)$ as the contribution to be assigned a tie in this case. A tie in the complete portion of the pedigree on both sides of course makes the unmodified contribution of $(\frac{1}{2})^{n+n'+1}(1+F_a)$. In dealing with partially complete pedigrees, care is of course necessary to see which pairs of random lines are eliminated from consideration by ties involving the complete part of the pedigree.

We are now in a position to consider more fully the method of calculating the average inbreeding of a group of animals. The ties between the sires and dams should be tabulated by the common ancestor involved. Certain of these animals may be found to be responsible for a large number of ties, others for only a few each. The pedigrees of those common ancestors which recur frequently should then be tabulated completely for a considerable number of generations in order to obtain a good estimate of their individual inbreeding by the method just discussed. For those which recur infrequently it is usually sufficient to assume an average degree of inbreeding equal to that of the breed as a whole at about the same time. The values adopted for the inbreeding of the various common ancestors (F_a) can then be used in the term $(1+F_a)$ in calculating the total coefficient. For example, the closest connection between the sire and dam of the bull Millionaire (79438), in Table I, is the bull Favourite (252). Favourite happens to be responsible for many more ties in Shorthorn pedigrees than is any other animal. As he came early in the history of the breed, his complete coefficient of inbreeding can be calculated without difficulty, and it turns out to be 19.2 per cent. Millionaire (79438) thus must be assigned a weight of $0.50\ (1+0.192)=0.596$ in finding the average degree of inbreeding of any tabulation of Shorthorns in which he is included.

The probable error must be rated up to the final value. If p is the proportion of ties observed, $q\ (=1-p)$ the proportion of pairs of lines which do not contain ties, and N is the number of pairs of lines compared, the probable error of F_x is $0.6745\sqrt{\frac{pq}{N}}\times\frac{F_x}{p}$ to a first approximation. For two-column pedigrees, N is of course simply the number of animals chosen from the breed. If four-column pedigrees are used, there are four possible ties in each pedigree, so that N is four times the number of animals. With eight-column pedigrees, N is 16 times the number of animals, etc. The probable error is reduced somewhat if partially complete pedigrees are used instead of ones in which all lines back of sire and dam respectively are purely random. There is no element of chance in the ties in the complete portion of such pedigrees, so that the contribution of those ties has no probable error. In applying the formula to partially complete pedigrees, p should include only ties involving random lines, and its probable error should be multiplied by the ratio of the portion of F_x, due to such ties to p.

These formulae give the probable error of the approximate formula for the particular animals chosen. Strictly, the probable error can be applied to a larger group of which these animals are a random sample only in case two-column pedigrees are used. As applied to single individuals, the formula gives the probable error of the approximate coefficient merely as a measure of the complete coefficient. It does

not, of course, give the probable error of the change in percentage of homozygosis. This is a function of the unknown number of Mendelian factors which were heterozygous in the foundation stock. The complete coefficient should give the decrease in heterozygosis very accurately for characters dependent on thousands of genes but with very little reliability in an individual case for a character dependent on only two or three genes.

RELATIONSHIP

The calculation of coefficients of relationship from random samples of pedigrees offers no additional complications of importance.

The presence of a tie between *single* random lines back of the two animals considered (X, Y) indicates a coefficient of $\frac{1+F_a}{\sqrt{(1+F_x)\,(1+F_y)}}$. A tie in a comparison of four-column pedigrees (four possible ties) contributes $\frac{1}{4}\left[\frac{1+F_a}{\sqrt{(1+F_x)\,(1+F_y)}}\right]$ to the coefficient, and similarly for larger pedigrees. In calculating the relationship of a large group to a particular animal (Y), the coefficient of inbreeding of that animal (F_y) should of course be obtained with a high degree of reliability. The coefficient (F_a) of common ancestors that frequently recur should be calculated with considerable reliability. The coefficient (F_x) for the animals of the group should be calculated once for all, preferably by the two-column method.

In this case, the denominator becomes a constant factor, and only the numerator varies for different ties. The reader will have no difficulty in extending the method to other sorts of cases, such as finding the correlation between random individuals of the breed, or within a specially choice section of the breed, or between two sections of the breed. The probable error can be calculated from the proportion of ties, and be rated up by the ratio of the coefficient to this proportion as in the case of the inbreeding coefficient.

The writers have made a number of tests of the reliability of the method. The average coefficient of inbreeding of the 64 Bates Duchesses, calculated from the complete pedigrees, was given as 40.9 per cent. The random method, using four-column pedigrees, gave 42.2 per cent with a probable error of 1.1 per cent. The observed difference (1.3 per cent) happens to be slightly greater than the probable error. It is evident that the enormously simpler approximate method gives a sufficiently accurate measure of the average inbreeding of the Duchesses as a group.

As an example of the application of the method to the inbreeding of an individual, it was found that 1,024 wholly random two-column samples of the pedigree of Favourite (252) gave a coefficient of 19.0 ± 0.52 per cent. The complete method gives 19.2 per cent.

FURTHER STUDIES ON ISOELECTRIC POINTS FOR PLANT TISSUE [1]

By WILLIAM J. ROBBINS, *Botanist*, and IRL T. SCOTT, *Assistant Plant Pathologist*, *Missouri Agricultural Experiment Station* [2]

INTRODUCTION

Previous papers by one of the writers (*5*, *6*)[3] have indicated that plant tissue—in its absorption of water, in its absorption and retention of acid and basic dyes, and in its effect upon the reaction of buffer mixtures in which the tissue is placed—acts much like an amphoteric colloid having an isoelectric point.

The so-called isoelectric point for potato-tuber tissue in the previous experiments seemed to be near P_H 6.0, for the mycelium of *Rhizopus nigricans* near P_H 5.0, and for *Fusarium lycopersici* near P_H 5.5. These points are apparently of importance in the physiology of the organisms because a low point in the growth of *R. nigricans* (*6*) and of *F. lycopersici* (*8*) is correlated with the so-called isoelectric point when the organisms are grown on media of different hydrogen-ion concentrations. This point is also apparently correlated with the ability of *F. lycopersici* to infect tomato plants (*8*) grown in soils of varying hydrogen-ion concentration.

In this paper the results of further experiments on the analogy between plant tissue and an amphoteric colloid with a definite isoelectric point are presented. As has been pointed out by Michaelis (*3*), an ampholyte placed in weak buffer mixtures changes toward greater alkalinity the reaction of those buffer mixtures that are more acid than the isoelectric point, and changes toward greater acidity the reaction of those buffer mixtures that are more alkaline than the isoelectric point. This is because the ampholyte acts as an acid in solutions more alkaline than the isoelectric point, and as an alkali in solutions that are more acid than the isoelectric point. At the isoelectric point, the reaction of the solution in which the ampholyte is placed remains unchanged. One of the writers has to some extent used this method of determining the isoelectric point with potato-tuber tissue (*5*), and the mycelium of *R. nigricans* (*6*). The previous experiments, however, were subsidiary to those on water absorption, dye absorption, and growth. The experiments reported here were designed to follow out more elaborately with plant tissue this method of determining the isoelectric point.

PROCEDURE

The general procedure was as follows. The plant tissue was added to a small quantity of a dilute buffer mixture, and the changes in reaction were measured at intervals until equilibrium was reached. If the tissue acts like an amphoteric colloid, with a definite isoelectric

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[3] Reference is made by number (italic) to "Literature cited," page 398.

point, it should change toward greater alkalinity the reaction of solutions that are more acid than the isoelectric point, and vice versa. The equilibrium point reached would depend on the relative quantities of tissue and buffer mixture, the reactive power of the tissue, and the strength of the buffer mixture. If an ampholyte were present in excess, the equilibrium point would correspond with the isoelectric point. If it were deficient, the equilibrium point would be more acid or more alkaline than the isoelectric point, depending upon which side the initial reaction lay.

The tissues used were potato-tuber tissue, root tips of Virginia soy beans, and the mycelial mats of three fungi: *Fusarium lycopersici*, *Gibberella saubinetii*, and *Fusarium oxysporum*.

The reaction of the solutions was measured electrometrically with the saturated calomel electrode, a modified Hildebrand type of bubbling hydrogen electrode, a Leeds-Northrup type K potentiometer, and type R galvanometer. The hydrogen electrode was a straight piece of platinum wire suitably coated with platinum black. A siphon bridge was used with a curved capillary for making contact with the solution in the electrode vessel, and with a wooden plug in the end in the saturated potassium chloride solution to minimize diffusion. When the wooden plug was placed in the end of the siphon in contact with the liquid in the electrode vessel, disturbances were produced which interfered with the determination of the E. M. F. The bridge was left in contact with the liquid in the electrode vessel during the entire time of the experiment. Some diffusion of the saturated potassium-chloride solution into the electrode vessel probably occurred, and, if it did, this may have been a disturbing factor in the experiments. The wooden plug and capillary, however, prevented much diffusion.

All determinations were made at temperatures which varied between 23.5° and 26.0° C. The light from an electric lamp shone on the tissue during the experiments.

EXPERIMENTAL DATA

POTATO-TUBER TISSUE

The potato-tuber tissue was prepared as described earlier (*5*), in disks about 1 cm. in diameter and 1 mm. thick. About 4 gms. were placed in 10 c. c. of 0.001 M. potassium dihydrogen phosphate, the reaction of which had been adjusted to the desired P_H by the addition of 0.001 M. potassium hydroxide. The electrode was surrounded by a basketwork of platinum to keep the potato disks from touching it. The electrode vessel consisted of the lower third of a 100 c. c. graduated cylinder. With the hydrogen bubbling continuously through the solution, the E.M.F. was read at intervals until an equilibrium point was reached. The tissue was then removed from the solution, and the E.M.F. determined. This was considered the final reading. In some cases a fresh lot of tissue was added to the solution in order to make certain that the equilibrium point reached was not due to the saturation of the tissue with the particular ion concerned.

The data for a typical pair of experiments performed on the same day are given in Table I. In experiment 12, a solution of P_H 6.32 became more alkaline, reaching a final equilibrium point of P_H 6.44. In experiment 13, a solution of P_H 6.69 became more acid, reaching an

equilibrium point of P_H 6.46. In experiment 12 (Table I), the P_H dropped from 6.32 to 6.26 2 minutes after the addition of the potato-tuber tissue, and at the end of 10 minutes it had reached a constant value of P_H 6.44. In experiment 13 (Table I), the addition of the potato had reduced the original P_H of 6.69 to 6.31 at the end of 3 minutes. At the end of 9 minutes, the Sörensen value of the solution had risen to 6.61 and then fallen slowly to 6.50 by the end of 60 minutes. The addition of a fresh lot of potato-tuber tissue to this solution caused a drop from P_H 6.50 to P_H 6.24 at the end of 6 minutes. At the end of 12 minutes after the fresh tissue was added the Sörensen value was 6.46, at which point it remained.

TABLE I.—*Data for two typical experiments with 4.5 gms. of potato-tuber tissue placed in 10 c. c. of 0.001 M. potassium dihydrogen phosphate, the initial reaction of which was adjusted with 0.001 M. potassium hydroxide and the reaction measured until equilibrium was reached*

Experiment 12		Experiment 13	
Time in minutes	P_H	Time in minutes	P_H
0	6.32	0	6.69
2	6.26	3	6.31
10	6.44	9	6.61
18	6.44	14	6.59
24	6.44	26	6.55
30	6.44	45	6.52
(a)	(a)	57	6.51
37	6.44	70	6.50
39	6.44	(b)	(b)
		76	6.24
		82	6.46
		91	6.46
		102	6.46
		(a)	(a)
		106	6.46
		109	6.46

[a] Tissue removed at this point.
[b] Added 4.5 grams fresh potato at this point.

The drop in Sörensen units a few minutes after the addition of the potato tissue to the solution, and the subsequent recovery, was noted in all the experiments summarized in Table II, except in experiments 3, 4, and 5. In these three experiments the initial P_H was 6.14 or less, and in all others 6.32 or greater. This immediate and temporary increase in acidity on the addition of the potato-tuber tissue to the dilute buffer mixtures is probably due to carbon dioxide introduced with the tissue and swept out in a few minutes by the constantly bubbling hydrogen.

TABLE II.—*Summary of experiments with potato-tuber tissue. Tissue placed in each case in 10 c. c. of 0.001 M. potassium phosphates, and reaction determined until equilibrium was reached*

Experiment No.	Initial P_H	Final P_H	Time in minutes	Amount potato in grams	Experiment No.	Initial P_H	Final P_H	Time in minutes	Amount potato in grams
1	6.54	6.39	145	4.1	8	6.67	6.46	63	4.3
2	6.32	6.02	119	4.1	9	6.33	6.16	165	4.5
3	5.85	6.20	72	4.0	10	6.33	6.37	33	4.5
4	6.10	6.48	116	4.4	11	6.69	6.38	90, 31	2×4.5
5	6.14	6.43	99	4.3	12	6.32	6.44	30	4.5
6	6.68	6.45	54	4.3	13	6.99	6.46	70, 30	2×4.5
7	6.32	6.44	55	4.3					

The writers conclude from the two experiments presented in Table I that potato-tuber tissue in 0.001 M. potassium phosphates acts like an amphoteric colloid with an isoelectric point of P_H 6.45. On the acid side of this point the tissue acts mainly as a base, taking up the phosphate ion chiefly; and on the alkaline side it acts mainly as an acid, taking up the potassium ion.

Most of the results in 11 other experiments confirmed those of the 2 just described. In Table II are summarized 13 experiments made with potato-tuber tissue by the methods which have been given. The initial P_H of the solution, the final P_H of the solution, the length of time the experiment was run, and the weight of the potato-tuber tissue are given for each experiment. In experiments 11 and 13, the original lot of tissue was replaced by a fresh lot. Experiments done on the same day on the same sample of potato tubers are bracketed together. An examination of the data in Table II shows that in 7 cases the final equilibrium point reached was P_H 6.43 to 6.46; in 3 additional cases P_H 6.37 to 6.39. In experiment 3 the final point reached was P_H 6.2. This probably represents, however, an equilibrium point due to a deficiency of ampholyte. The solution in experiment 3 was the most acid one used and would necessitate a greater absorption to bring its reaction to the isoelectric point than would solutions such as were used in experiments 4 and 5. If a fresh lot of tissue had been added to the solution used in experiment 3 the Sörensen value would probably have been increased. In only two cases were the results contrary to the conclusion that potato-tuber tissue in 0.001 M. potassium phosphates acts like an amphoteric colloid with an isoelectric point near P_H 6.4. In experiments 2 and 9 the addition of potato-tuber tissue to solutions of P_H 6.32 and 6.33 lowered the P_H instead of raising it. Whether this is due to experimental errors, to variations in the potato tissue, or to some other cause is an unsolved problem.

Other experiments than those summarized in Table II were made with potato-tuber tissue. The results, however, were considered misleading because from 25 to 50 c. c. of solution was used in a 150 c. c. beaker and the hydrogen electrode was unprotected. This larger amount of liquid and the larger container did not permit the complete and rapid removal of the carbon dioxide by the stream of hydrogen. The potato disks crowding together on one side of the beaker prevented the ready mixing of the solution with the possible development of a local zone the reaction of which would be different from that in the vicinity of the electrode. Experiments in which potato-tuber tissue was placed in 50 c. c. of 0.001 M. potassium phosphates of initial reaction of P_H 5.73, 6.37, 6.48, and 6.97, showed final Sörensen values, after 6 to 7 hours, of P_H 6.30, 6.78, 6.70, and 6.86, respectively. Hydrogen was bubbled through the solutions part of the time during these experiments, and part of the time the potato stood quietly in the solutions. These high values are no doubt due to the presence of carbon dioxide, which kept the reaction of the tissue, or a part of it, on the acid side of the isoelectric point, and induced a continued absorption of the phosphate ion as in the experiments with soy-bean root tips described later in this paper.

The results of the experiments given in Table II agree fairly well with those performed earlier by one of the writers (*5*) on the effect of buffer mixtures on water absorption and dye absorption by potato-

tuber tissue. They show that potato-tuber tissue affects the reaction of 0.001 M. potassium phosphates much the same as would an amphoteric colloid with a definite isoelectric point. The so-called isoelectric point found in the present experiments is more alkaline than that found in the earlier experiments. A more dilute buffer mixture, and the potassium phosphates instead of the sodium phosphates, were used in the present experiments. It is interesting to note that Stiles and Jørgensen (*10*) found that potato-tuber tissue neutralized both dilute hydrochloric acid and dilute potassium hydroxide.

SOY-BEAN ROOT TIPS

Virginia soy beans were germinated between filter papers in pans, and a centimeter or two of the root tips was cut off. From 24 to 60 such root tips were placed in 10 c. c. of the 0.001 M. potassium phosphates. The electrode used was of the same type as that used in the experiments with potato, but it was not protected with the platinum basketwork, as the root tips, being smaller, were less likely to strike it.

The results of the experiments with soy-bean root tips are summarized in Table III. The results are much less uniform than with potato. Solutions of initial P_H of 6.68, 6.69, and 6.78 were made more acid. Solutions of initial P_H of 5.82 and 6.20 were made more alkaline. In three cases, solutions of P_H 6.32 or 6.33 became more alkaline, and in four cases more acid. The lowest Sörensen unit reached from the alkaline side was P_H 5.89, and the highest from the acid side was P_H 6.44. Considering experiments performed on a single day, such as 1 and 2, 5 and 6, and 9 and 10, the root tips affected the solutions as an ampholyte would be expected to affect them. The majority of the experiments indicate that the isoelectric point for soy-bean root tips lies in the vicinity of P_H 6.20 to 6.44.

TABLE III.—*Summary of experiments with Virginia soy-bean root tips. Tissue in each case placed in 10 c. c. of 0.001 M. potassium phosphates and reaction determined until equilibrium was reached*

Experiment No.	Time in days from beginning of germination	Initial P_H	Final P_H	Time in minutes	Weight of tissue in grams	Experiment No.	Time in days from beginning of germination	Initial P_H	Final P_H	Time in minutes	Weight of tissue in grams
1	7	6.20	6.43	45, 25	2×0.7	6	7	6.32	6.19	100	1.5
2	7	6.78	6.42	34, 54, 38, 108	4×0.7	7	5	6.32	6.38	116	1.5
						8	5	5.82	6.18	112	1.5
3	4	6.32	6.34	127	0.8	9	6	6.33	6.44	93	1.3
4	5	6.68	6.19	180, 49	2×0.9	10	6	6.69	6.49	150, 18	2×1.3
5	7	5.82	6.13	39, 25, 20, 23	4×1.5	11	7	6.33	5.96	191	1.3
						12	8	6.32	5.89	164	2.3

The writers do not know why the results of successive experiments with the soy-bean root tips are not duplicates. One factor may be the age of the root tips; another may be the fact that meristematic tissue was being dealt with as well as partially or completely differentiated tissue, the proportions varying in the different lots of root tips.

Some extremely interesting observations were made on the effect of the acidity due to carbon dioxide on the changes in reaction pro-

duced by the root tips. In the experiments in which hydrogen was bubbled continuously through the liquid in which the soy-bean root tips were placed, the final reaction reached from the acid side (Table III) was never more than P_H 6.44. If the root tips were allowed to stand undisturbed in the liquid, the P_H measured colorimetrically quickly approached 5.9, at which point it remained constant. If the reaction of the solution were then determined electrometrically, it was found to be near P_H 6.7. This is illustrated by the following experiment.

Sixty root tips of Virginia soy beans, totaling about 3.2 gms., were placed in 25 c. c. of 0.001 M. potassium phosphates in a 150 c. c. beaker and allowed to stand quietly. The reactions of the solutions were read colorimetrically. Within 10 minutes a solution of original P_H 5.6. had changed to P_H 5.9, and it remained unchanged in reaction for 90 minutes, during which time determinations of the hydrogen-ion concentration were made colorimetrically at intervals. After 60 minutes a solution of original P_H 6.1 had changed to P_H 5.9. After 90 minutes the reactions of the two solutions, which were P_H 5.9 colorimetrically, were determined electrometrically. The values were P_H 6.72 and 6.85, respectively. The solutions were removed from the electrode vessels and the reaction measured colorimetrically. The results were P_H 6.7 and 6.9, respectively.

These results were evidently due to the carbon dioxide produced by the respiration of the root tips. Standing quietly in the solutions in the beakers, carbon dioxide given off by the root tips accumulated in the solutions and kept the reaction near P_H 5.9. This point, as indicated by the majority of the data in Table III, is acid to what the writers have called the isoelectric point. The absorption of the phosphate ion therefore continued. When the electrometric determination was made, the carbon dioxide in the solution was swept out by the hydrogen, and the reaction in the absence of the carbon dioxide was found to be P_H 6.7 to 6.9. If the carbon dioxide had not been present, the reaction would probably have slowly changed in both solutions to approximately P_H 6.4, at which point the change would have ceased. Evidently acidity produced by carbonic acid affects the relative amounts of cations and anions absorbed, just as does the acidity produced by phosphoric acid.

MYCELIAL MATS OF GIBBERELLA SAUBINETII

This fungus, as well as the two others described later in this paper, was grown on the mineral nutrient solution used by one of the writers (*8*) with a total concentration of 0.066 M. monobasic and dibasic potassium phosphate and an initial P_H of 5.0. The mycelial mats were removed from the nutrient solutions and thoroughly washed with 0.2 M. cane-sugar solution or redistilled water. The excess liquid was pressed out of the mats, which were placed in the dilute buffer mixtures, where readings were taken, with the hydrogen bubbling continuously until equilibrium was reached.

This strain of *Gibberella saubinetii* was one isolated by B. B. Branstetter from corn. The results obtained with this organism are summarized in Table IV, and Figure 1 shows curves with the P_H of the solutions on the ordinate and the time on the abscissa.

TABLE IV.—*Summary of results with mycelium of Gibberella saubinetii. Tissue in each case placed in solution indicated and reaction measured until equilibrium was reached*

Experiment No.	Weight of mycelium	Age of mycelium	Kind of buffer solution used	Quantity of buffer used	Initial P_H	Final P_H	Time required to reach equilibrium
	Grams	*Days*		*C. c.*			*Minutes*
1	5.67	15	0.002 M. Na phosphates	50	4.03	6.14	60
2	4.40	15	0.001 M. Na phosphates	50	4.37	6.05	65
3	4.49	16	0.001 M. KH phthalate+H_3PO_4	25	3.15	6.23	21
4	1.51	16	0.001 M. Na phosphates	25	5.88	6.25	19
5	1.88	11	0.001 M. Na phosphates	25	6.68	6.21	77
6	4.25	14	0.001 M. Na phosphates	50	7.88	6.34	126
7	4.65	16	0.001 M. Na phosphates	50	7.42	6.36	103

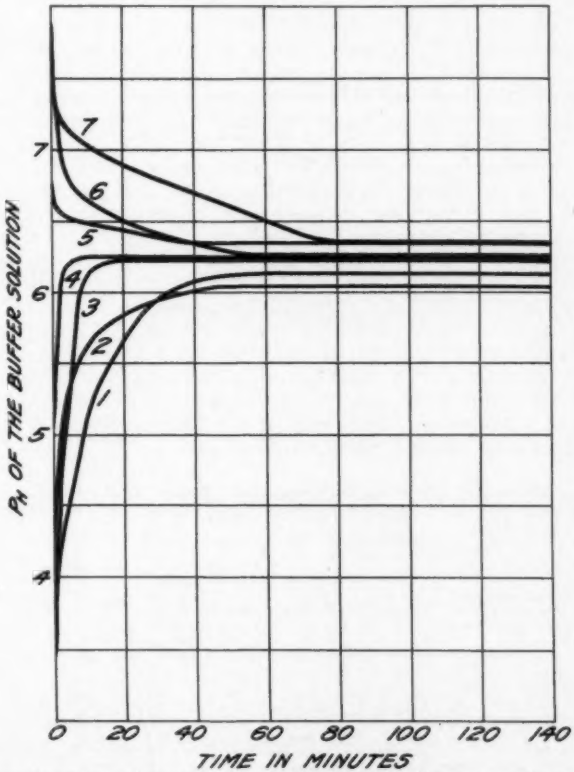

FIG. 1.—Curves showing the effect of the mycelium of *Gibberella saubinetii* upon the reaction of dilute buffer mixtures P_H of the buffer mixture is plotted against the time. The numbers on the curves refer to the experiments summarized in Table IV

The effect of the mycelium of *G. saubinetii* upon the dilute buffer mixtures was similar to that produced by an ampholyte with an isoelectric point near P_H 6.2. Solutions of initial P_H 3.15, 4.03, 4.37,

and 5.88, became more alkaline because of the mycelium; solutions of initial P_H 7.88, 7.42, and 6.68 became more acid. The lowest final P_H reached from the alkaline side was 6.21, and the highest P_H reached from the acid side was 6.25. The curves in Figure 1 show very clearly how the mycelium of this fungus brought the reaction of the buffer mixtures from both the acid and alkaline side toward P_H 6.2.

For the most part, the action of the fungus mycelium in changing the reaction of a solution was greater and more rapid than that of the potato-tuber tissue or soy-bean root tips. This was also true of the other two fungi used. It may be due to the more ready access of the liquid to the hyphae of the fungus rather than an actual difference in the action of the cell contents.

The greater rapidity with which equilibrium was reached in the phthalate buffer mixture is of interest. Comparing experiments 2 and 3, it is evident that approximately the same amount of mycelium required 65 minutes to shift the reaction of 0.001 M. sodium phosphates from P_H 4.37 to 6.05 and only 21 minutes to make a greater change in a 0.001 M. phthalate buffer mixture. This may be due to the cell contents of the fungus being already partially saturated with the phosphate ion, since it was grown in a nutrient solution containing phosphates, while its reactivity for the phthalate ion would be entirely unsatisfied.

The isoelectric point found in these experiments for *Gibberella saubinetii* is somewhat more alkaline than that which might be deduced from the growth experiments and infection experiments carried on with this organism by Hopkins (2). He found a minimum in the growth curve at P_H 5.5 to 6.0 when *G. saubinetii* was grown in solutions of various acidities and a minimum infection of wheat seedlings in soils with artificially adjusted reactions at P_H 5.5. These minima have been interpreted by one of the writers (*5*) to be due to the decreased water absorption which occurs at the isoelectric point. The solutions, however, used by Hopkins were complete nutrient solutions and much stronger in buffer salts than those the writers have used. Moreover, the strain of the fungus was not the same. These facts may account for the difference.

TABLE V.—*Summary of results with mycelium of Fusarium lycopersici. Tissue in each case placed in solution indicated, and reaction measured until equilibrium was reached*

Experiment No.	Weight of mycelium	Age of mycelium	Kind of buffer solution used	Quantity of buffer solution used	Initial P_H	Final P_H	Time required to reach equilibrium
	Grams	*Days*		*C. c.*			*Minutes*
1	0.71	8	0.01 M. KH phthalate	10	3.94	4.88	30
2	2.14	14	0.002 M. Na phosphates	50	4.03	5.50	70
3	1.50	8	0.001 M. KH phthalate	10	3.95	5.47	11
4	1.50	8	0.001 M. KH phthalate	10	3.95	5.41	16
5	5.08	7	0.001 M. Na phosphates	50	4.55	5.41	56
6	4.04	9	0.001 M. Na phosphates	50	5.43	5.48	54
7	0.635	5	0.001 M. Na phosphates	10	6.66	5.46	39
8	1.63	4	0.001 M. Na phosphates	10	7.20	5.52	34
9	[1] 0.94	16	0.001 M. Na phosphates	25	6.02	6.16	20
10	4.92	7	0.001 M. Na phosphates	50	7.20	6.00	60

[1] Autolyzed.

MYCELIAL MATS OF FUSARIUM LYCOPERSICI

The results obtained with the mycelial mats of *Fusarium lycopersici* are summarized in Table V. In Figure 2, curves of the results are given with the P_H of the solutions on the ordinate and time on the abscissa.

The mycelium of *F. lycopersici* affects the buffer mixtures used like an ampholyte with an isoelectric point at P_H 5.5. Solutions of initial reaction P_H 3.94, 3.95, 4.03, 4.55, and 5.43 became more alkaline because of the presence of this fungus, while solutions of initial P_H 7.20 and 6.66 became more acid. The results of experiment 9 can be overlooked in drawing conclusions, because the 16-

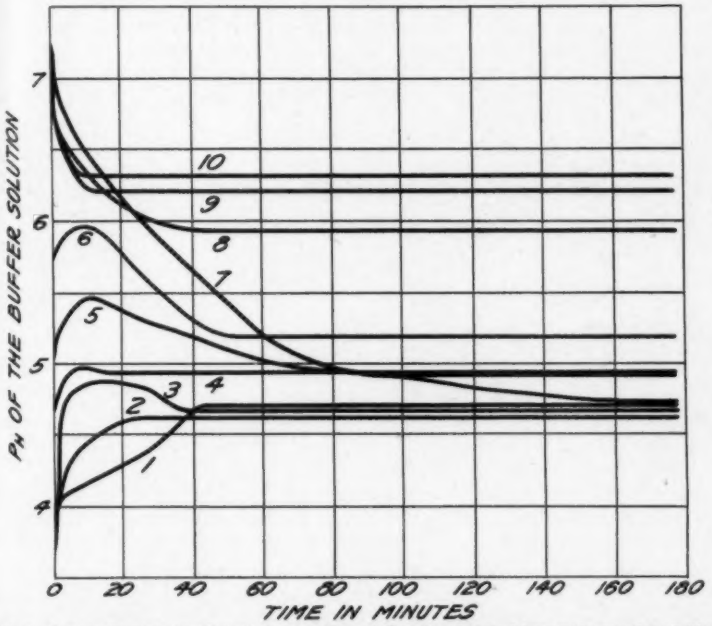

FIG. 2.—Curves showing the effect of the mycelium of *Fusarium lycopersici* upon the reaction of dilute buffer mixtures. The P_H of the buffer mixture is plotted against time. The numbers on the curves refer to the experiments summarized in Table V

day old mycelium used in this experiment was badly autolyzed. Disregarding this experiment, the lowest P_H reached from the alkaline side was P_H 5.52, and the highest from the acid side was P_H 5.50.

Again the much greater rapidity of action of the mycelium in changing the reaction of phthalate buffer mixtures, as compared to the phosphate solutions occurred. In experiment 4 it required 16 minutes for 1.50 gms. of the mycelium to change an 0.01 M. phthalate solution of P_H 3.95 to a P_H of 5.41; while, in experiment 2, 2.14 gms. of mycelium required 70 minutes to make a smaller change in the reaction of a 0.002 M. phosphate buffer mixture. This difference in speed of action is brought out clearly by the much steeper slope of the curve for experiment 4 as contrasted with that for experiment 2, as shown in Figure 2.

The changes in reaction in experiment 6 did not follow the regular order of the balance. Instead of proceeding uniformly toward an equilibrium point, the mycelium changed the reaction to above P_H 5.50, then to a point below that value, followed by a second oscillation toward greater alkalinity before the reaction settled to an equilibrium point at P_H 5.48. This is shown by the curve for experiment 6 in Figure 2. The initial reaction of the solution in experiment 6 was more nearly the isoelectric point than any other used. This fact may be connected with the peculiar action of the mycelium in this experiment.

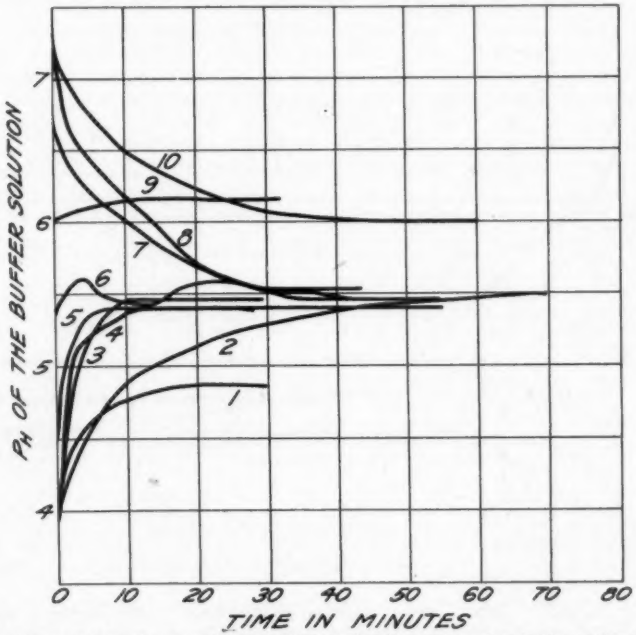

FIG. 3.—Curves showing the effect of the mycelium of *Fusarium oxysporum* upon the reaction of dilute buffer mixtures. The P_H of the buffer mixture is plotted against time. The numbers on the curves refer to the experiments summarized in Table VI

The isoelectric point found for the mycelium of *Fusarium lycopersici* in these experiments agrees very well with that found earlier in studies on the influence of hydrogen-ion concentration on dye absorption (6), and on the growth of the same strain of this organism (8).

MYCELIAL MATS OF FUSARIUM OXYSPORUM

The results of experiments with *Fusarium oxysporum* are summarized in Table VI and Figure 3.

The mycelium of *F. oxysporum* acts much like an ampholyte with an isoelectric point near P_H 4.9. Solutions of initial P_H 3.80, 4.02, 4.04, and 4.73 became more alkaline because of the presence of the mycelium; while solutions of initial P_H 7.36, 7.10, 6.94, 5.70, and 5.06 became more acid. The highest final P_H reached from the acid side was 4.94, and the lowest from the alkaline side was 4.71.

In the curves of Figure 3, a peculiarity of the results of some of the experiments (3, 4, 5, and 6) with this fungus is evident. In all four of these experiments the initial effect of the mycelium was to produce an alkalinity greater than the final equilibrium point, forming humps on the curves.

TABLE VI.—*Summary of results with mycelium of Fusarium oxysporum. Tissue in each case placed in the solution indicated, and reaction measured until equilibrium was reached*

Experiment No.	Weight of mycelium	Age of mycelium	Kind of buffer solution used	Quantity of buffer solution used	Initial P_H	Final P_H	Time required to reach equilibrium
	Grams	*Days*		*C. c.*			*Minutes*
1	1.03	17	0.01 M. KH phthalate	10	4.02	4.70	40
2	1.13	17	0.01 M. KH phthalate	10	4.04	4.63	38
3	3.17	9	0.001 M. Na phosphates	50	3.80	4.64	52
4	0.50	15	0.0005 M. Na phosphates	25	4.73	4.94	19
5	0.90	17	0.001 M. KH phthalate, KOH	10	5.06	4.71	166
6	1.46	9	0.001 M. Na phosphates	10	5.70	5.195	54
7	1.39	9	0.001 M. Na phosphates	10	7.36	4.92	101
8	2.282	11	0.001 M. Na phosphates	50	6.94	5.94	58
9	1.50	11	0.001 M. Na phosphates	10	7.10	6.21	15
10	0.47	11	0.001 M. Na phosphates	5	7.10	6.31	12

DISCUSSION

The experiments reported here show that under the conditions of these experiments, plant tissue acts much like an ampholyte with a definite isoelectric point. For potato-tuber tissue this point is near P_H 6.4, for the mycelium of *Gibberella saubinetii* near P_H 6.2, for that of *Fusarium lycopersici* near P_H 5.5, and for *Fusarium oxysporum* near P_H 4.9. For the root tips of soy beans it is in the neighborhood of P_H 6.2 to 6.4, but further work is necessary to define it more exactly, if possible.

The decided difference in the so-called isoelectric points for these three closely related fungi suggests the possibility of using this method or one similar to it as an aid in identification.

The experiments also emphasize the importance of the acidity produced by carbonic acid. If the writers' interpretation of the experiments is correct, the accumulation of this acid can profoundly affect the absorption of ions by the cell, and probably, also, other physiological processes which depend upon reaction with the isoelectric point as the critical one. Most of the isoelectric points reported here are in the zone in which carbonic acid can affect the reaction. This suggests the importance of considering the carbon-dioxide content of the cell contents when the hydrogen-ion concentration of plant tissue is determined. Apparently most determinations of the hydrogen-ion concentration of plant tissues are now made on the extracted sap with no attention paid to the carbon-dioxide content of the juice. Yet the acidity due to carbon dioxide may be as important in plant juices as in the blood of animals. Its accumulation in the tissues of higher plants at night when the stomates are closed might throw the reaction across the isoelectric point or move it further away with possible profound physiological effects.

The methods used in the experiments reported here need further refinement; and a study of the effect of various conditions, such as

other buffer mixtures, light, age, death, temperature, and dissolved materials, should be made. The main thesis, however, that plant tissue acts much like an amphoteric colloid with definite isoelectric points appears clear, substantiated as it is by earlier work by the writers. Stearn and Stearn (*9*) have come to somewhat similar conclusions in their work on the staining of bacteria.

At the same time several facts also show that plant tissue does not act completely like a simple protein such as gelatin. The overlapping of the final equilibrium points noted in the experiments described here with all the tissues, except that of *F. lycopersici*, would not be expected with gelatin. It must be remembered, however, that protein and other materials of plant tissue are inclosed in a cellulose wall, and that the reaction in the cell may not be that of the solution in which it is immersed. The shifting of the so-called isoelectric point caused by ions other than the hydrogen and hydroxyl ions, noted in the earlier experiments on water absorption by potato-tuber tissue (*5*) in dilute buffer mixtures, would not occur if we were dealing with the isoelectric point of gelatin. The overlapping of the zones of absorption and retention of basic and acid dyes in plant tissue placed in solutions of different hydrogen-ion concentrations, noted by Rohde (*7*) and by one of the writers for living tissue, and by Naylor[4] for dead tissue does not occur with pure gelatin. The absorption of both anions and cations on both sides of the isoelectric point must occur, else organisms could not exist as they do over a comparatively wide range of hydrogen-ion concentration. This is not what would be expected from a substance like gelatin.

What happens in the simple buffer mixtures used in the experiments reported in this paper is probably much similar to what Rohde found to be true for the influence of hydrogen-ion concentration on the absorption of dyes, and pictured so clearly in the curves he presents. On the acid side of what we have called the isoelectric point, both anions and cations are taken up, the former, however, more rapidly and in greater amount. The further we move toward greater acidity away from the isoelectric point, the more anions and the fewer cations are absorbed. On the alkaline side of the isoelectric point the situation is reversed.

In plant tissue we are probably dealing with several amphoteric colloids which have different isoelectric points. Owing to their amphoteric properties, these substances may interact with one another and form one or more amphoteric compounds which may be necessary to life. That the constituents of the cell do not all act alike, at least when dead, has been shown by Naylor. With sections of root tips he has found that the cytoplasm, resting nucleus, chromosomes, and nucleolus possess different isoelectric points.

We may also be considering not the isoelectric point but the point of maximum undissociated ampholyte molecules, the ρ maximum of Michaelis (*3*). The isoelectric point, as shown by Michaelis, is not affected by salt formation, but the ρ maximum is affected by salt formation. The hydrogen-ion concentration for it may coincide with the isoelectric point, or may lie on either side of it, depending upon the dissociation constants of the salts formed. This possibility is sug-

[4] NAYLOR, E. E. THE EFFECT OF THE HYDROGEN-ION CONCENTRATION UPON THE STAINING OF PLANT TISSUE BY BASIC AND ACID DYES. 1924. [Unpublished thesis, Univ. Mo.]

gested by the differences found in the position of the isoelectric point for potato-tuber tissue, as defined by the water absorption in different buffer mixtures.

Whatever may be the ultimate complete and correct explanations of these writers' data, the conception of the plant cell as an amphoteric colloid is one which will prove increasingly valuable. Pearsall and Priestley (*4*), and Weber (*11*), have suggested theories for the existence or formation of meristem based upon such a conception. Arrhenius (*1*), in studying the ammonia, nitrate and total nitrogen content of soils of varying natural hydrogen-ion concentration, found that while the total nitrogen was rather constant in amount, the ammonia content was at its maximum in the acid soils and the nitrate in the alkaline soils. When plotted against the P_H of the soil, the two curves for nitrates and ammonia content intersect near P_H 6.5. This is what we should expect, if plants absorbed ions as they are taken up by an ampholyte with an isoelectric point near P_H 6.5. Nitrate, as an anion, would be absorbed to the greatest extent from soils more acid than P_H 6.5, and ammonia, a cation from soils more alkaline than P_H 6.5. The maximum amount of nitrate would be left in alkaline soils and the maximum amount of ammonia in the acid soils. The isoelectric points given in this paper for potato-tuber tissue and soy-bean root tips are close to this value.

Speculation, however, seems to the writers as somewhat futile, on the basis of the experimental facts they now have. More complete and more careful data from a variety of viewpoints are needed. The writers are convinced, however, that most of the difficulties in problems of salt absorption, water absorption, "permeability," toxicity, and antagonism can be solved, and the mass of data concerning them unified by considering plant tissue as an amphoteric colloid which reacts with ions according to chemical laws, rather than as a system of osmotic chambers with membranes of changeable permeability.

SUMMARY

When potato-tuber tissue was placed in small quantities of 0.001 M. buffer mixtures of the potassium phosphates, and the reaction was determined electrometrically with the hydrogen bubbling continuously, solutions of P_H 6.5 or greater became more acid, and solutions of P_H 6.14 or less became more alkaline. Solutions of P_H 6.32 or 6.33 became more alkaline in some cases and more acid in others.

In the majority of cases the equilibrium point reached with potato-tuber tissue under the conditions just referred to was P_H 6.37 to 6.46. The lowest equilibrium point reached from the alkaline side was P_H 6.02, and the highest from the acid side was P_H 6.44.

When allowed to stand quietly for part of the time in larger quantities of the buffer mixtures, the final reaction reached, as determined electrometrically, was P_H 6.3 to 6.86.

When soy-bean root tips were placed in small quantities of 0.001 M. buffer mixtures of the potassium phosphates, and the reaction was determined electrometrically with the hydrogen bubbling continuously, solutions of P_H 6.68 or greater were made more acid and solutions of P_H 6.2 or less were made more alkaline. Solutions of P_H 6.32 or 6.33 became more acid in some cases and more alkaline in others.

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In the majority of cases the equilibrium point reached with soy-bean root tips under the foregoing conditions was P_H 6.18 to 6.49. The lowest equilibrium point reached from the alkaline side was P_H 5.89, and the highest from the acid side was P_H 6.44.

When allowed to stand quietly in larger quantities of the buffer mixtures, the equilibrium point reached, as determined colorimetrically, was P_H 5.9; as determined electrometrically, P_H 6.72 to 6.85.

When the mycelium of *Gibberella saubinetii* was placed in small quantities of dilute buffer mixtures, and the reaction determined electrometrically, solutions of P_H 5.88 or less became more alkaline, and solutions of P_H 6.88 or greater became more acid. The lowest equilibrium reached from the alkaline side was P_H 6.21, and the highest from the acid side was P_H 6.25.

When the mycelium of *Fusarium lycopersici* was treated in the same way, solutions of P_H 5.43 or less became more alkaline, and solutions of P_H 6.66 or greater became more acid. The lowest equilibrium point reached from the alkaline side was P_H 5.52, and the highest from the acid side, with one exception, was P_H 5.50.

When the mycelium of *Fusarium oxysporum* was treated in the same way, solutions of P_H 4.73 or less became more alkaline, and solutions of P_H 5.06 or greater became more acid. The lowest equilibrium point reached from the alkaline side was P_H 4.71, and the highest from the acid side was P_H 4.94.

It is concluded that, under the particular conditions of these experiments, these plant tissues act much like amphoteric colloids, with isoelectric points as follows: For potato-tuber tissue at P_H 6.4; for soy-bean root tips (variety Virginia) at 6.2 to 6.44; for the mycelium of *Gibberella saubinetii* at 6.2; for the mycelium of *Fusarium lycopersici* at 5.5; and for the mycelium of *Fusarium oxysporum* at 4.9.

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